

Diagnostic tests in ragweed hay fever

A comparison of direct skin tests, IgE antibody measurements, and basophil histamine release

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In 87 patients with both spring and fall hay fever symptoms the radioallergosorbent test (RAST) technique for specific IgE antibodies to ragweed was compared with basophil histamine release and direct intradermal skin testing by the threshold dilution technique. The three techniques gave good agreement except with the least-sensitive patients, some of whom had a positive skin test but undetectable histamine release or IgE antibodies. Twenty-one patients who were highly sensitive to ragweed as measured by all three techniques were followed without specific immunotherapy. There was significant agreement between the level of positivity of all three tests and the symptom index obtained during the ragweed season. In 14 of the 21 patients there was a significant correlation between daily ragweed pollen counts and daily symptom indexes during the season. On the other hand, among the 16 least-sensitive patients (as judged by histamine release) the correlation between daily ragweed pollen counts and symptom indexes was significant in only 3 patients. Other significant allergens could not be identified in the latter group, and the cause of their symptoms is not clearly identified but appears not to be ragweed. The RAST is a quantitative technique that gives diagnostically useful information in ragweed hay fever, although not significantly different from basophil histamine release or carefully performed skin testing. The convenience to the patient may, however, offer a noticeable advantage.

Precise diagnosis of the causative agent in apparently allergic symptoms has always been difficult in the practice of allergy and has usually required careful and detailed historical investigation by the specialist in allergy. Immunologic methods of measuring responses to allergens in vitro have been undergoing development in recent years¹⁻⁷ but require repeated assessment in comparison with clinical data and the time-honored technique of direct skin testing.

Among allergists, skin testing some years ago fell into disrepute as a quantitative procedure. In 1936 Walzer⁸ noted in a review: "Many writers are now taking a hypercritical and even derogatory stand concerning the value of

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results in skin testing." In criticism of skin tests the following points have been noted: (1) Positive skin tests to an allergen may be found in individuals who disclaim symptoms to that allergen.⁹ (2) Individuals with characteristic symptoms and positive mucosal challenge tests to a given allergen may nevertheless be skin-negative.¹⁰ (3) Identical readings in terms of wheal size may be obtained from extracts that are 10 or even 100 fold different in concentrations by dilution.¹¹ (4) The same extract injected into different anatomic skin sites will give different reaction sizes.¹⁰ (5) Extracts of the same allergen may vary considerably in potency from one batch to another.¹² (6) Extracts stored at the extreme dilutions required for skin testing may deteriorate rapidly.¹² (7) The prior use of sympathomimetic or antihistamine drugs by the patient may reduce the size of the skin test.¹³

Despite these considerations, it is often recommended that patients be classified into 3 or 4 broad groups on the basis of the size of the reaction to a particular concentration of allergen and to initiate desensitization treatment with smaller doses in the patients with larger reactions.¹³ The implication here is that the patient who is more sensitive by skin test is more likely to have an untoward allergic reaction to injections of extract. Despite the third criticism above, Dorfman and Efron¹¹ noted that when the upper limit of dilution was approached with a specific allergen there were rather sharp decreases in wheal size with successive dilutions. Plateaus in the size of reaction were noted principally when the concentration was in excess of that required to give a rather large wheal (8.0 mm. or larger) with pseudopods.

Our interest in this subject began when we first began to use direct intradermal skin tests to assess the relative potency of fractions prepared from ragweed pollen extract.^{14, 15} In an attempt to minimize the known sources of variation in skin testing, the following precautions were taken: (1) Fractions were diluted in a buffer containing 0.03 per cent serum albumin as a stabilizer. (2) Only the skin of the back was employed where a large area of equal reactivity was available. (3) Disposable syringes and needles were employed to eliminate problems with residual allergen from earlier uses. (4) Tests to be compared were done at the same sitting. (5) No medication was allowed the subject in the previous 24 hours. (6) 0.05 ml. was injected so that the amount injected might be more accurately measured. (7) Testing was carried through dilutions weak enough to give a negative or trace reaction. (8) The smallest amount required to give a 2+ (1.0 cm. wheal, 2.0 to 3.0 cm. erythema) reaction was considered an end point.

Under these circumstances, reproducible results were obtained that clearly distinguished between one fraction and another. While no precise estimate of accuracy was attempted, threefold differences in activity seemed detectable without duplicate testing. When leukocyte histamine release techniques became available (the histamine is now known to come almost entirely from the basophils), entirely consonant results were obtained with the same ragweed fractions, suggesting that the two techniques were reflections of the same basic process.^{15, 16} Subsequently, in an evaluation of antigen E as a standard for ragweed pollen extract, we measured the potency of a number of commercial

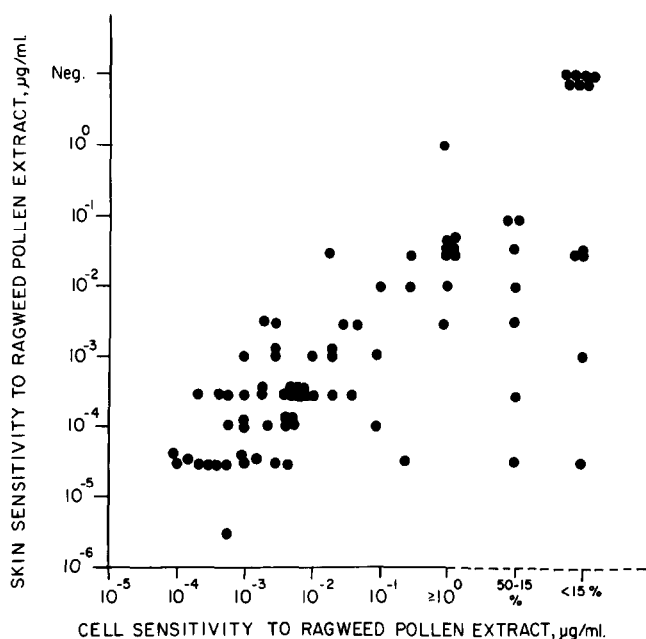


FIG. 1. Cell sensitivity and skin sensitivity to whole ragweed pollen extract.

extracts by both skin test and histamine release. With our technique for skin testing the two methods gave virtually identical results with a correlation coefficient of better than 0.9.¹⁷ As the determination of cell sensitivity by histamine release has consistently over a number of years predicted the severity of disease in ragweed-sensitive patients,^{2, 18-23} it therefore seemed important to re-examine the dictum that skin tests have little value in predicting the severity of disease.

With the RAST developed by Wide, Bennich, and Johansson,³ it is possible to measure specific IgE antibodies without the dangers of passive transfer to human subjects or the complexities of passive transfer to human leukocytes in vitro. This test has shown promise as a diagnostic measure in allergy⁴⁻⁷ and deserves further comparison with traditional skin tests and with histamine release.

When a new study of immunotherapy was being organized in 1970, patients who volunteered for the study had both cell sensitivity determinations by histamine release and careful quantitative skin tests performed using the same highly potent ragweed pollen extract. The results in 87 patients confirmed the close relationship between skin tests and histamine release.^{24, 25} Sera were stored on these patients pending the eventual measurement of specific IgE antibodies. In those patients who were good histamine releasers and strongly positive by skin test, the ability of these two techniques to predict the severity of disease was confirmed in those patients who went untreated in the placebo group. A report demonstrating the ability of measurements of IgE antiragweed antibody also to predict disease in these patients has already appeared.²⁶

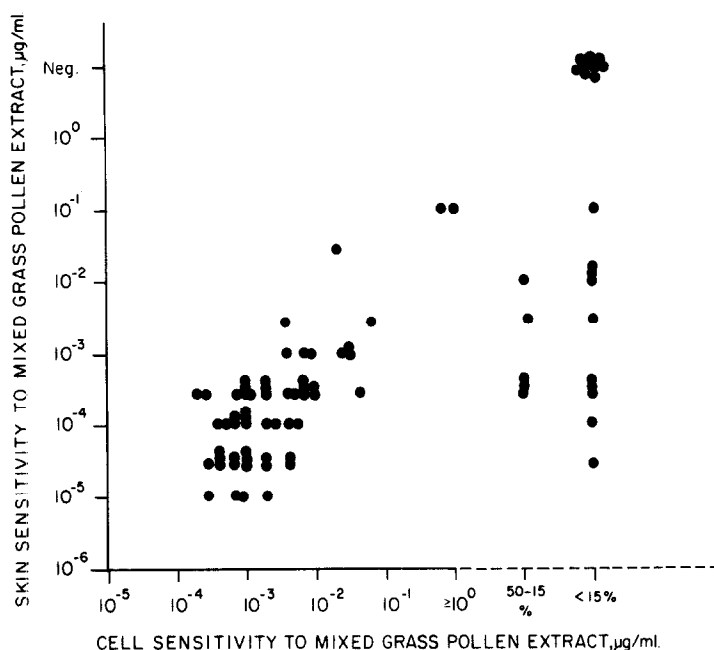


FIG. 2. Cell sensitivity and skin sensitivity to mixed grass pollen extract.

Those patients who had poor release of histamine and also had a low level of skin test sensitivity represented special cases and were considered not to have the diagnosis of hay fever confirmed. These patients were subsequently found to have a low level of IgE anti-ragweed antibodies and were not included in our therapeutic study. They were, however, given placebo injections and followed through the hay fever season with symptom diaries in the same fashion as the more sensitive patients in the therapeutic study. When daily symptom reports were compared with pollen counts, these observations cast doubt on the original impression of ragweed hay fever in this group and will be discussed more fully below.

MATERIALS AND METHODS

Antigens

Whole short ragweed pollen extract. Whole short ragweed pollen extract was prepared by Hollister-Stier Labs., Spokane, Washington, for the committee on standardization of allergens of the National Institute of Allergy and Infectious Diseases. It was furnished as a lyophilized extract containing 27 µg antigen E per 10,000 protein nitrogen units (PNU).

Whole grass extract. Whole grass extract was a gift of Dr. David G. Marsh and contained a mixture of grass pollens in the following 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.

Patients

Histamine release data on these patients have already been reported.²⁴ Individuals who responded to a newspaper story asking for volunteers with hay fever were sent a simple

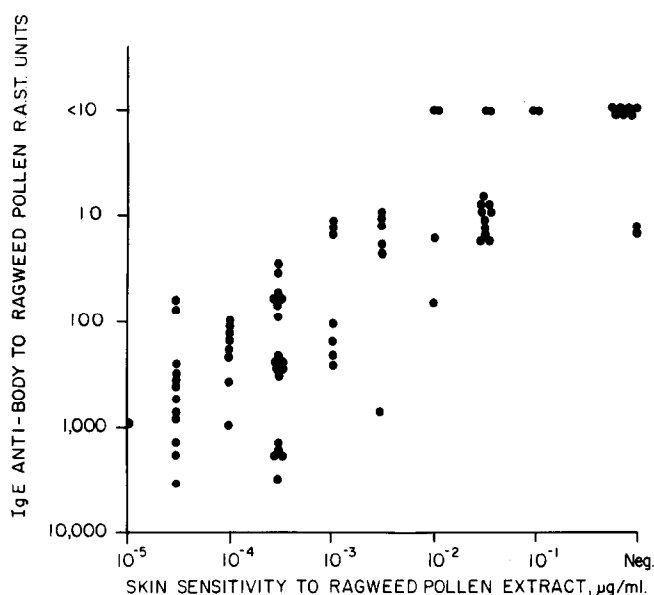


FIG. 3. Skin sensitivity to ragweed pollen extract and specific IgE antibody to ragweed pollen extract measured by RAST.

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 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.

Symptom evaluation

This was carried out by techniques previously reported.^{2, 21} Briefly, a history form was filled out by each patient for each 12 hour period of the ragweed season, recording the duration of sneezing, rhinitis, conjunctivitis, and cough. Antihistamine use was also recorded. The forms were collected weekly and the reported symptoms scored numerically and entered into a computer program. This yielded a symptom score for each patient (or each group of patients) for each day of the ragweed season.

Skin testing

Intradermal skin tests were performed on the back employing the threshold dilution technique. Tenfold dilutions of the ragweed and grass whole pollen extracts were injected intradermally with a disposable No. 27 needle and plastic syringe introducing 0.05 ml. of extract starting with a solution of 10^{-4} μ g N per milliliter (0.01 PNU per milliliter). After 15 minutes reactions were graded as described by King Norman, and Connell.¹⁴ If negative, more concentrated solutions were tried until a good positive reaction (2+ or more) was obtained or until a concentration of 10 μ g N per milliliter (1,000 PNU per milliliter) was tried with no reaction. On the other hand, if the initial test was positive, less concentrated solutions were assayed until a minimal positive (trace) or a negative test was obtained. The least dilution required for a 1+ or 2+ test was considered the end point. When this end point seemed to fall between two dilutions, i.e., the stronger gave a 3+ or 4+ reaction and the weaker a trace of negative reaction, the end point was considered intermediate between the two dilutions and an interpolated value half way (on a logarithmic scale) between the two dilutions was entered as an end point.

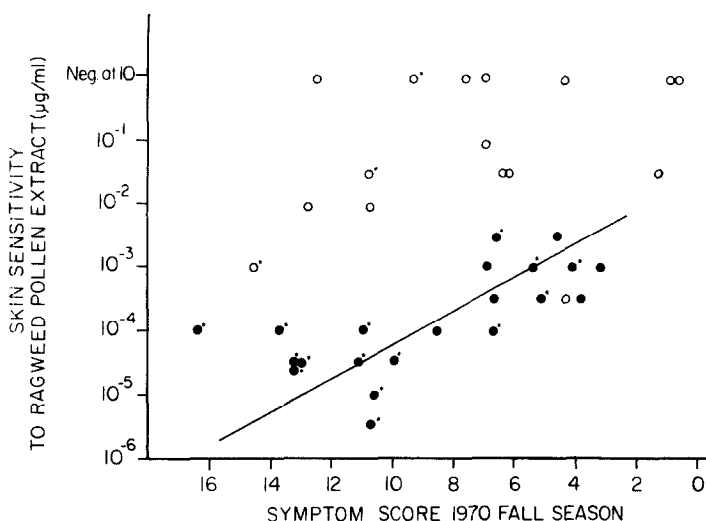


FIG. 4. Skin test to ragweed pollen extract and average daily symptom scores. Closed circles indicate group highly sensitive by skin test; open circles indicate group poorly sensitive by histamine release; asterisks indicate patients with significant correlation between daily pollen counts and daily symptom scores.

Specific IgE antibody (RAST)

IgE antibody against ragweed antigen was measured by the method of Wide and associates.³ Details of the procedure were described in a previous paper.²⁶ Briefly, ultrafine Sephadex (Pharmacia, Uppsala, Sweden) was activated by cyanogen bromide and coupled with fraction D of ragweed pollen extract.^{14, 15} Specifically purified rabbit anti-IgE antibody described in the previous paper²⁶ was labeled with ^{125}I by the method of Klinman and Taylor.²⁷ The IgE antibody in the test samples was estimated from a standard curve obtained with our reference standard serum. Since the P-K titer of the reference serum was 1:4,000, the IgE antibody level in this serum was considered to be 4,000 units per milliliter and the IgE antibody levels in the test samples were expressed in terms of these units.

RESULTS

Fig. 1 compares the threshold of skin test reactivity with the 50 per cent end point of histamine release in the 87 patients under study. Seven patients showed some but less than 50 per cent release of histamine. As it was not possible to give a 50 per cent release cell sensitivity figure in these patients, they are grouped on a line that reads 50 to 15 per cent release at the right of the graph. All 7 of these patients had positive skin tests but at a level indicating a lesser degree of sensitivity than the average for the group who released 50 per cent or more of their cellular histamine. Twelve patients showed less than 15 per cent histamine release; 7 of them were negative by skin test, and the others had lower levels of skin reactivity than the 75 per cent release group.

Fig. 2 shows a similar comparison of skin test and histamine release data in the same patients with grass pollen extract. A similar pattern is noted. The 5 patients with some but less than 50 per cent histamine release have a less than average level of sensitivity by skin test. Of the 19 patients with virtually no

TABLE I. Maximal per cent histamine release of leukocytes tested in TACM and TACM plus 5 per cent human serum

	Patient No.						
	1	2	3	4	5	6	7
Grass Group I, TACM	8	5	7	31	26	2	38
Grass Group I, TACM + serum	11	6	8	51	26	1	54
Ragweed AgE, TACM	0	0	5	35	—	1	8
Ragweed AgE, TACM + serum	0	3	20	50	—	1	8

release of histamine to grass extract, 9 were negative by skin test and the other 10 as a group had less than average levels of skin test reactivity.

With both ragweed and grass extracts the end point in skin test titrations showed about a tenfold greater sensitivity than the 50 per cent end point in histamine release.

As noted here and in an earlier publication,¹⁹ the leukocytes of some patients showed very little histamine release at any antigen concentration even though they were skin test positive. Patients with these characteristics have been shown to have significantly less severe clinical manifestations of allergy than patients with fully reactive cells.¹⁹ Recently May and associates²⁸ reported data suggesting that some or all of these patients' leukocytes failed to react fully because they were suspended in a suboptimal medium: the addition of serum to the medium increased the response. We tested this proposition with the leukocytes of 7 patients; aliquots of the patients' cells were suspended either in the usual buffer or this buffer with the addition of 5 per cent human serum from nonallergic blood type AB donors. The results are shown in Table I. The patients whose cells in TACM alone had less than 10 per cent histamine release did not show significantly more release in 8 of 9 experiments; the exception went from 5 to 20 per cent release but still showed a minimal response. In 3 of 4 instances in which the leukocytes in TACM released 26 to 38 per cent there was some enhancement of release, as we have noted previously.²⁹ The increase averaged only about 13 per cent and did not transform the curves to the more usual response of highly sensitive patients, i.e., 70 to 100 per cent maximal histamine release.

Fig. 3 presents a comparison between skin tests to ragweed pollen extract and specific IgE antibodies to ragweed. The agreement is as good as that noted between cell sensitivity and skin tests. Several patients are noted in whom IgE antibodies were undetectable yet who had undoubtedly positive skin tests at a low level. As the limit of sensitivity of the measurement of IgE antibody is 10 units, it is still possible that these patients have IgE antibodies but at levels so low as to be missed by this technique. The 2 patients with negative skin tests but detectable IgE antibodies to ragweed cannot be explained from our present knowledge.

As noted in an earlier publication, many of these patients entered into a therapeutic study and received ragweed pollen extract, so that clinical symptomatology can be considered only in those patients who received placebo treatment.²⁶ There were 21 patients highly sensitive by the 3 tests reported here who received placebo treatment only. A significant correlation between cell sensitivity

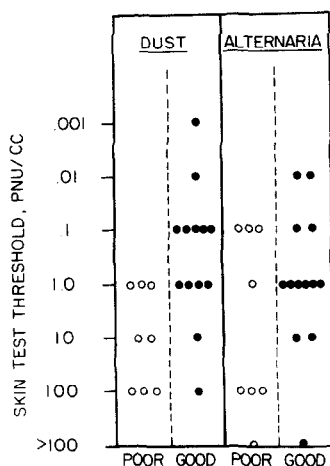


FIG. 5. Skin tests to dust and Alternaria. Closed circles indicate group highly sensitive to ragweed, and open circles indicate group poorly sensitive to ragweed.

and symptomatology was noted in this group, as has regularly been noted in other groups of highly sensitive patients. We have at the same time reported a significant correlation between specific IgE antibodies to ragweed extract and symptoms in the same patients.²⁶ On the other hand, no comparison is possible between symptoms and cell sensitivity in the patients with little or no histamine release. There were 16 such patients who were carefully followed after placebo injections. Seven of them showed no response to an intradermal test at a concentration of 10 μ g N per milliliter (1,000 PNU per milliliter). The remaining 9 were positive by skin test but mostly at a level lower than that observed among the patients with readily observable histamine release. An attempt could be made to correlate symptoms with the low-level skin tests in these patients. Fig. 4 shows the relationship between skin test and symptom scores in all the patients (both highly sensitive and poorly sensitive) followed after placebo treatment and distinguishes the patients with poor release from those with good release by symbol. Among those patients who were highly sensitive by histamine release a significant degree of correlation exists between skin sensitivity and symptoms. On the other hand, most of the patients who were poorly sensitive by histamine release have higher symptom scores than would be predicted by the foregoing relationship.

Several possibilities can be cited to explain the apparent discrepancy in the poorly sensitive patients: (1) Sensitivities other than ragweed are the principal determinants of their symptoms. (2) These patients have ragweed hay fever by mechanisms other than IgE-mediated histamine release. (3) They have nonallergic disease.

In an attempt to examine the first possibility, during the fall of 1971, some of these patients were examined for other sensitivities by skin test. Tests were performed for molds and dust. Not all of the patients originally studied were still available, but 8 patients from the low-releasing group and 13 from the high-releasing group could be studied. Tests were performed with extracts of Alter-

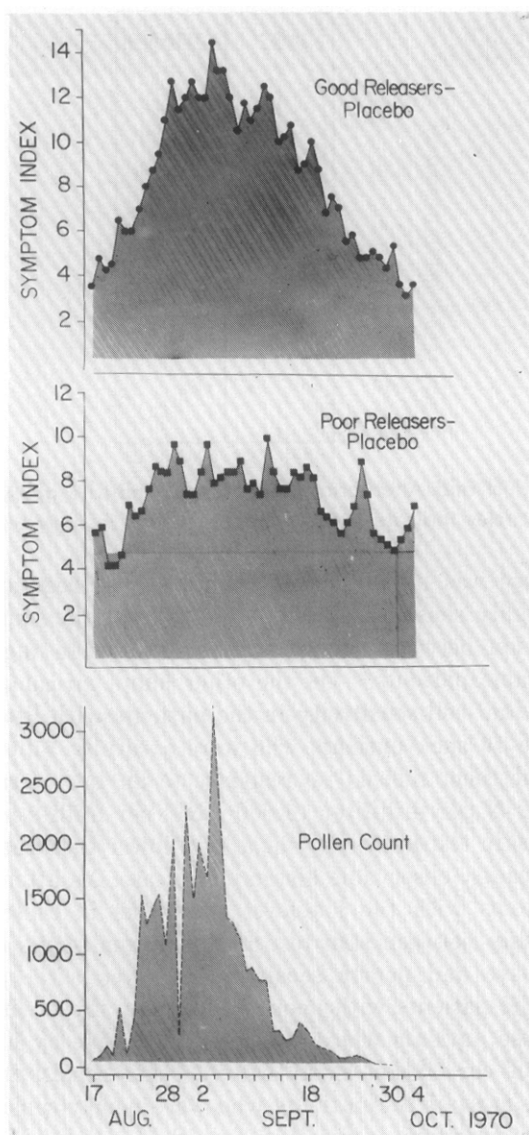


FIG. 6. Pollen count and average daily symptom scores for 1970.

naria, *Aspergillus*, *Hormondendrum*, and dust. *Aspergillus* and *Hormondendrum* were either nonreactive or slightly positive only at 10 μ g N per milliliter and hence were not considered further. Fig. 5 shows the results with dust and *Alternaria* extracts. With both antigens the degree of reactivity was noticeably lower in the poor-release group than in the good-release group. These findings fail to furnish any evidence that mold and dust antigens are greater determinants of symptomatology in the poor-release group than they are in the good-release group.

We then considered the question of whether the poor-release patients had

TABLE II. Correlation between daily pollen counts and symptom scores

Highly sensitive patients				Poorly sensitive patients			
Patient	No. obs.	Coefficient	Significant	Patient	No. obs.	Coefficient	Significant
C. L.	23	+0.784	< 0.01	B. H.	49	+0.631	< 0.01
H. S.	21	+0.759	< 0.01	R. R.	49	+0.610	< 0.01
C. N.	21	+0.730	< 0.01	R. H.	49	+0.449	< 0.01
W. L.	49	+0.701	< 0.01	N. W.	28	+0.348	Not sig.
M. B.	47	+0.620	< 0.01	R. W.	49	+0.299	< 0.05
B. T.	35	+0.619	< 0.01	L. McG.	49	+0.223	Not sig.
W. M.	49	+0.605	< 0.01	S. E.	49	+0.111	Not sig.
F. L.	41	+0.506	< 0.01	G. D.	49	+0.040	Not sig.
E. B.	42	+0.501	< 0.01	W. B.	45	+0.038	Not sig.
M. L.	49	+0.488	< 0.01	A. G.	49	-0.025	Not sig.
R. S.	49	+0.473	< 0.01	R. D.	49	-0.063	Not sig.
G. H.	49	+0.470	< 0.01	T. E.	49	-0.127	Not sig.
M. T.	49	+0.441	< 0.01	C. S.	49	-0.154	Not sig.
S. Z.	49	+0.423	< 0.01	M. M.	49	-0.179	Not sig.
R. V.	49	+0.355	< 0.05	V. T.	49	-0.227	Not sig.
M. B.	49	+0.241	Not sig.	F. F.	32	-0.401	< 0.05
B. S.	36	+0.229	Not sig.				
J. D.	49	+0.153	Not sig.				
C. W.	49	+0.058	Not sig.				
C. S.	49	-0.138	Not sig.				
C. T.	41	-0.394	Not sig.				

symptoms in a pattern that allowed one to confirm that their disease was indeed related to ragweed pollen exposure. Fig. 6 shows daily averages of symptom scores in the two groups in comparison with semiquantitative pollen counts obtained from an Ogden rotoslide pollen sampler placed at a suburban point in Baltimore. Whereas there is a good relationship between pollen count and symptoms in the highly sensitive group, the poorly sensitive group was symptomatic both at the beginning and at the end of the season when pollen counts were low, and their symptoms showed little seasonal rise.

In order to examine the pattern of symptoms patient by patient in the two groups, a correlation coefficient was calculated for daily pollen count and daily symptom scores in each patient. A value of +1.0 would indicate perfect correlation in this case, a value of 0 no correlation, and a value of -1 a perfect inverse correlation. Table II shows these coefficients arrayed in order of decreasing values in the two groups. In the highly sensitive group significant positive correlation coefficients were noted in 14 of the 21 patients. In the poorly sensitive group only 3 of 16 patients showed such a correlation. Indeed, 10 of the patients showed correlations near 0 or negative.

We interpret such results to indicate that most of the poorly sensitive patients were symptomatic through other causes than ragweed pollen. In Fig. 4 the patients with significant correlations between pollen count and symptoms are marked by an asterisk. Even the 3 patients among the poorly sensitive group with good correlations are far more symptomatic than would be predicted on the basis of their skin tests. In these patients the possibility of symptoms due to ragweed but determined by mechanisms other than IgE-mediated histamine release might be raised, but there is little or no evidence that such mechanisms actually can or do play a role.

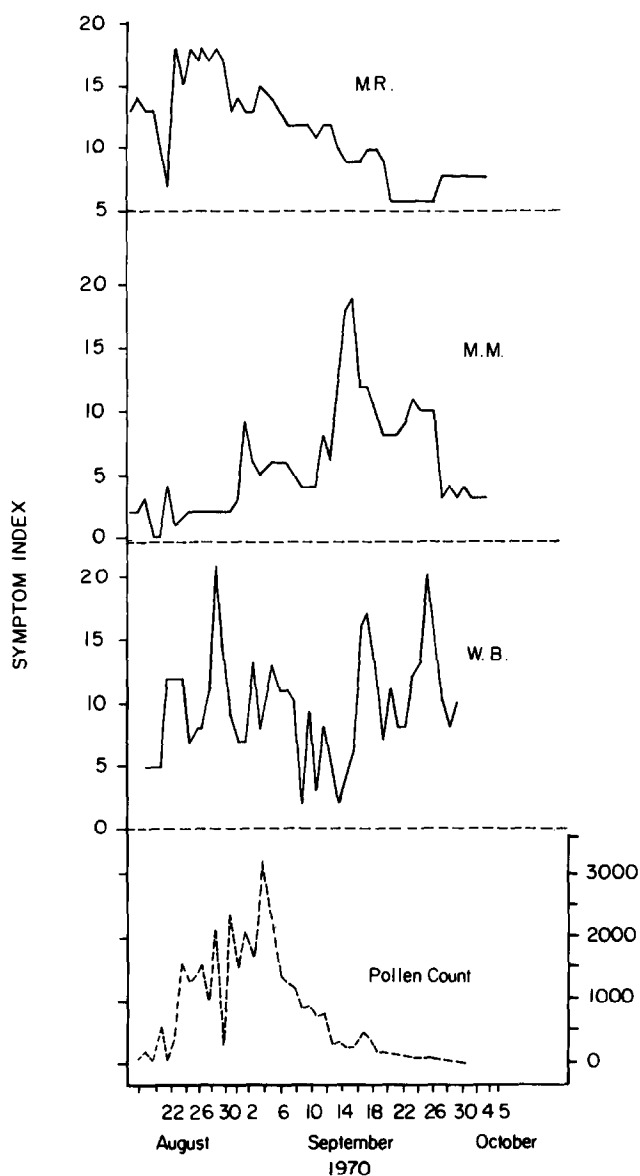


FIG. 7. Individual daily symptom scores for 3 patients from the poorly sensitive group.

It is also possible that the annual decline of IgE antibodies commonly observed during spring and summer in many ragweed-sensitive patients is much exaggerated in these people and actually reaches undetectable levels between seasons, only to be restimulated to a clinically important degree during the season. Unfortunately, serum was not collected immediately after the season from the patients in whom the divergence between symptoms and measures of sensitivity raises this question.

If the poorly sensitive patients are responding to allergens other than rag-

weed, we have not been able to demonstrate their allergy by skin testing for mold and dust antigens. Their symptoms may arise from the nonallergic influences often cited as contributory in patients with rhinitis symptoms, i.e., emotions, infections, vasomotor instability, etc.

Fig. 7 shows examples of the pattern of daily symptom scores in 3 of these patients to show the variability observed. Patient M. R. had a distinct peak in symptoms early in August that was definitely diminishing in severity at the height of pollination. Patient M. M. was relatively asymptomatic during the maximum of pollination and became symptomatic only later. Patient W. B. was symptomatic both before and after pollination but was better when ragweed pollen was at its height. Several others (not shown) were continuously symptomatic throughout the period of observation. The poorly sensitive group observed here are quite mixed in the pattern of disease they show. In studies of desensitization they would serve to confuse the issue seriously if they were included because of skin tests that are positive but only at a low level.

DISCUSSION

In this study direct skin tests performed with due care to technique and to good quality reagents gave results that quantitatively were comparable with two more recently developed immunologic techniques. Direct measurement of IgE antibodies in serum by the RAST has the apparent advantage of measuring antibody but not other aspects of the biologic reaction leading to a wheal-and-erythema response. Histamine release *in vitro* depends on the amount of specific IgE antibodies fixed to the surface of the basophilic leukocytes but is also subject to influences on, and individual variations in, the chain of intracellular events that lead to histamine secretion.²⁶ Skin tests are subject to such influences and further subject to variability based on the patients' responsiveness to mediators, anatomic skin sites, drug use, possibly hormonal state, etc.

From our data it would appear that in actual practice such influences are not major and that the biologic tests and the chemical tests will perform similarly in diagnosis. The choice of technique to use as a diagnostic aid therefore becomes a matter of relative expense and convenience to patient and physician. Direct skin tests in this context have the advantage of immediacy and simplicity. They are readily performed with simple materials and give answers immediately. They represent some discomfort to the patient and some risk of untoward reactions, although adequate attention to the relative potency of various extracts and care to eliminate errors of dosage reduces risk to an almost negligible point. Basophil histamine release, as currently performed in our laboratory, requires an excessively large sample of fresh blood for the study of more than a few allergens. While this can be scaled down, the need for highly trained technicians is nevertheless considerable. Automation might reduce the effort involved and the size of the specimen to practical levels, but the requirement for processing freshly drawn blood in less than an hour seems unlikely to be circumvented.

The RAST for specific IgE antibodies can be performed on relatively small amounts of stored serum and thus becomes quite convenient for the patient.

Results can be obtained in a day or two. As currently performed, the test requires considerable technical skill, but a relatively large number of specimens can be processed simultaneously. A considerable investment in a gamma counter is entailed.

From our data, we believe that these diagnostic tests are best used to make quantitative predictions of the severity of disease. In any such test there will be borderline cases in which the decision that a particular allergen plays a role in the patient's disease will be difficult. In these cases other sources of information, mostly clinical, will be needed to make an intelligent decision. In patients with such borderline tests, who are nevertheless highly symptomatic, other causes of disease, both allergic and nonallergic, should be sought. In view of such borderline instances it seems futile to attempt to set a cutting level for such tests, above which all patients will have disease and below which no patients will have disease. Only consideration of the level of positivity in conjunction with the other clinical information will allow a realistic diagnostic decision. For these reasons, the skilled clinician knowledgeable in allergic syndromes will continue to be required for adequate interpretation of improved quantitative diagnostic tests.

If the biologic tests (skin test and basophil histamine release) are to be used quantitatively, carefully standardized allergenic extracts will be required. At present, except for ragweed extract in which antigen E can be used as a standard, standardization can be accomplished only by comparison of an unknown extract with a standard, carefully preserved in the lyophilized state to maintain activity. Standardization for determination of specific IgE antibodies, on the other hand, requires the use of reference sera that are tested with each batch of unknown sera. Reproducible and meaningful results with the RAST technique probably also depend on the use of extracts with adequate amounts of the specific allergenic proteins present. Excessive amounts of inert materials appear to interfere with the coupling of allergen to the insoluble particulate and consequent loss of sensitivity. In this study we have employed a partially purified extract of ragweed to obviate this difficulty.

Our results indicate that direct skin testing has been unnecessarily denigrated as a quantitative technique. With proper methods and well-standardized materials the threshold of response is as useful in predicting symptoms as two sophisticated laboratory techniques. Standardization and proper storage of reagents to maintain potency seem to be the main desiderata in the quantitative use of skin tests.

If our results with ragweed allergy are confirmed with other allergens, it seems likely that tests for specific IgE antibodies will gain considerable acceptance not because of an innate superiority in furnishing quantitative data or a more strict relationship to actual disease than biologic tests, but because of the simple convenience to patients and the obvious lack of risk. The technique is too sophisticated to be performed readily in physicians' offices but could be performed efficiently in a centralized laboratory in which the volume of tests performed would justify efforts at standardization and the expensive equipment

involved. In the meantime it is of the utmost importance to work out standards for the allergenic extracts to be employed in skin testing and more accurately to determine those conditions of storage that will maintain adequate potency.

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