

Quantitative studies of cutaneous hypersensitivity: The prevalence of epicutaneous flare reactions to allergenic pollen extracts

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The flare reactions produced by epicutaneous tests with 68 undiluted allergenic pollen extracts were measured in 550 allergic patients. Skin test reactions ≥ 2 , ≥ 5 , ≥ 10 , ≥ 20 , and ≥ 30 mm in diameter, respectively, were detected in approximately 67%, 22%, 10%, 3%, and 1% of the 34,700 skin tests. With the Kolmogorov-Smirnov difference test, the cumulative frequency of reaction diameters and log_e-transformed diameters of all reactions and reactions to individual allergenic extracts differed significantly ($p \leq 0.01$) from a normal distribution. The ability to identify specific differences between reactions to closely related pollen extracts was evaluated. Specific reactions could be reliably identified with ≥ 10 mm diameter flares. This arbitrary conservative threshold was used to estimate the relative prevalence of positive reactions to each allergenic extract. Seven allergenic extracts elicited the first quartile of all positive reactions. Thirteen, 18, and 30 allergenic extracts, respectively, were needed to elicit the second, third, and fourth quartiles of all positive reactions. Reactions to amphiphilous, as well as anemophilous, pollens were detected. Skin test reactions to grasses were more prevalent than reactions to weeds and trees. The most informative allergenic extracts for the detection of patients who exhibited a positive reaction to any extract were from red fescue-grass pollens, mesquite, short ragweed, red clover, and timothy-grass pollens. (J ALLERGY CLIN IMMUNOL 1989;84:465-74.)

Percutaneous reactions to aeroallergens are prevalent in the U.S. population.¹ The Second National Health and Nutrition Examination Survey² revealed that scratch skin tests with eight common allergenic extracts elicited one or more ≥ 10.5 mm flare-reaction diameters in 20.2% of 16,204 participants. The four aeroallergen pollen extracts included in this important study elicited reactor prevalences that ranged from 4.4% for Bermuda grass to 10.2% for rye-grass. This article focuses on three additional aspects of cutaneous reactions to allergenic pollens: (1) the distribution of the size of flare reactions, (2) the size of a "positive" skin test reaction, and (3) the relative importance of different pollens. The flare reactions produced by scratch skin tests with undiluted allergenic extracts of 68 pollen allergens were evaluated in 550 patients.

The size, distribution, and specificity of the flare reactions were used to deduce that a ≥ 10 mm diameter flare does provide a reasonable conservative criteria for a positive skin test. This arbitrary criterion was used to describe the relative prevalence of specific reactions to the allergenic pollen extracts.

METHODS Allergens

The allergenic extracts were obtained from commercial suppliers (Greer Laboratories, Lenoir, N.C.; Hollister-Stier, Seattle, Wash.; and Allergy Laboratories of Ohio, Dayton, Ohio) or prepared locally as a 1/20 or 1/40 wt/vol extract in 50% glycerin and used throughout the period of study. Tables I and II list the allergenic extracts used. In Table I, the order, family, genus, species, and common names of six pollens from plants belonging to Gymnospermae division and 12 pollens from plants belonging to the Liliopsida class of the Angiospermae division are listed. In Table II, 50 additional pollens from plants belonging to other classes of the Angiospermae division are listed.³⁻⁶ Although most plant species from which the extracts were prepared are anemophilous or wind pollinated, amphiphilous species in which insect (entomophilous) or animal (zoophilous) vectors contribute to pollination were also included. The nonanemophilous or amphiphilous plant pollens are

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TABLE I. Gymnospermae and Angiospermae Liliopsida plant-pollen sources

Order	Family	Genus and species	Common name
*	Pinaceae	<i>Pinus virginiana</i>	Scrub pine
		<i>Pinus taeda</i>	Loblolly pine
		<i>Pinus echinata</i>	Shortleaf pine
		<i>Pinus palustris</i>	Longleaf pine
*	Cupressaceae	<i>Cupressus sempervirens</i>	Summer cypress
		<i>Chamaecyparis thyoides</i>	White cedar
Arecales	Arecaceae	<i>Phoenix dactylifera</i>	Date palm
		<i>Chamaerops humilis</i>	European palm
Cyperales	Poaceae	<i>Cynodon dactylon</i>	Burmuda
		<i>Sorghum halepense</i>	Johnson
	Phalarideae	<i>Anthoxanthum odoratum</i>	Sweet vernal
	Hordeae	<i>Lolium</i> sp	Ryegrass
	Tripsaceae	<i>Zea mays</i>	Corn
	Festuceae	<i>Dactylis glomerata</i>	Orchard
		<i>Poa</i> sp	Blue
		<i>Festuca rubra</i>	Red fescue
		<i>Agrostis stolonifera</i>	Redtop
	Agrostideae	<i>Phleum pratense</i>	Timothy

The common names of the 23 amphiphilous plants in Tables I and II that are not dependent on wind pollination are presented in boldface type; mesquite, red clover, alfalfa, acacia, and melaleuca are zoophilous, and the remaining amphiphilous plants are entomophilous.

*Gymnospermae families.

identified in Tables I and II. The outcome of tests with the amphiphilous pollen extracts are identified in Tables III and IV.

Skin tests

A solid bi-barbed needle was used to elicit scratch (epicutaneous) tests with each undiluted allergenic extract. The physical characteristics of the bi-barbed needle, the method of testing, and the precision of the experimental measurements of the flare reaction have been previously described.⁷ Differences in antigen potency variation during the time of testing were minimized by the use of undiluted allergenic extracts. Potency-related variation in the intensity of the allergenic stimulus yields maximum variation in reaction size across the steep portion of the dose-dependent sigmoid response curve.⁸ The stimulus intensity provided by epicutaneous and percutaneous tests with undiluted allergenic extracts lies near the upper extreme of the sigmoid curve, where extract potency and skin test device-related variation produce the least variation in reaction size. This precaution diminishes the chance that changes in allergen potency might alter estimates of reaction prevalence during the time of study.

Each patient was tested with each undiluted allergen, selected concentrations of methacholine, and a saline control. The maximum methacholine used for skin testing was 1777 mmol/L, and serial twofold dilutions contained 888, 444, or 222 mmol/L of methacholine. Crossed perpendicular diameters of the flare-and-wheal reaction were measured with a millimeter ruler 15 to 20 minutes after the cutaneous challenge. The averages of the respective wheal-

and-flare reaction diameters were recorded. Skin tests in the initial 147 patients were limited to one to two methacholine concentrations. Skin tests with three or more methacholine concentrations were available in 434 patients and were used to estimate methacholine responsiveness and sensitivity⁸ from, respectively, the midpoint of the sigmoid dose-response curve and the methacholine concentration needed to elicit a flare reaction equivalent to the estimate of responsiveness. The pollen-flare reactions, saline and methacholine control reactions, estimates of methacholine responsiveness and sensitivity, relevant demographic, and all available clinical information were transferred to electronic media before evaluation of the observations. The reactions to the pollen allergens are summarized in this article.

Subjects

The decision to perform skin tests on each patient was based on the exposure history and/or the presence of symptoms and objective physical findings consistent with an immediate hypersensitivity disease. Observations were available in 581 patients. The skin tests were reviewed, and 31 (5.3%) patients with dermatographism, manifested by equivalent-sized positive reactions to allergens and the saline control and/or apparent suppression of the expected flare reactions to methacholine, were deleted from the subject sample. This process yielded 37,400 measurements of skin test flare reactions produced by 68 allergenic pollen extracts in 550 patients. The subjects were predominantly female (63.1%), white (87.4%) adult (median and average age $\pm$ SD, 34 and 36.4 $\pm$ 14.0 years; range, 12 to 79

TABLE II. Angiospermae Magnoliopsida plant pollen sources

Order	Family	Genus and species	Common name
Hamamelidales	Platanaceae	<i>Platanus occidentalis</i>	Sycamore
	Hamamelidaceae	<i>Liquidambar styraciflua</i>	Sweet gum
Urticales	Ulmaceae	<i>Ulmus crassifolia</i>	Cedar elm
	Moraceae	<i>Morus rubra</i>	Red mulberry
Juglandales	Juglandaceae	<i>Carya illinoensis</i>	Pecan
		<i>Carya glabra</i>	Hickory
		<i>Juglans nigra</i>	Black walnut
Fagales	Fagaceae	<i>Quercus alba</i>	White oak
		<i>Quercus velutina</i>	Black oak
		<i>Quercus stellata</i>	Post oak
		<i>Quercus virginiana</i>	Live oak
		<i>Quercus rubra</i>	Red oak
		<i>Fagus grandifolia</i>	Beech
	Betulaceae	<i>Betula nigra</i>	River birch
		<i>Alnus serrulata</i>	Tag alder
Casuarinales	Casuarinaceae	<i>Casuarina equisetifolia</i>	Australian pine
Caryophyllales	Chenopodiaceae	<i>Chenopodium album</i>	Lamb's-quarter
	Amaranthaceae	<i>Amaranthus retroflexus</i>	Pigweed
Polygonales	Polygonaceae	<i>Rumex acetosella</i>	Sheep sorrell
Malvales	Malvaceae	<i>Hibiscus aculeatus</i>	Hibiscus
Salicales	Salicaceae	<i>Salix discolor</i>	Pussy willow
		<i>Populus alba</i>	Silver poplar
		<i>Populus tremuloides</i>	Aspen
Rosales	Rosaceae	<i>Rosa</i> sp	Rose
		<i>Prunus persica</i>	Peach
Fabales	Mimosoideae	<i>Albizia julibrissin</i>	Mimosa
		<i>Trifolium pratense</i>	Red clover
		<i>Prosopis juliflora</i>	Mesquite
		<i>Acacia</i> sp	Acacia
	Papilionoideae	<i>Medicago sativa</i>	Alfalfa
Myrtales	Myrtaceae	<i>Eucalyptus globulus</i>	Eucalyptus
		<i>Melaleuca leucadendron</i>	Melaleuca
Cornales	Cornaceae	<i>Cornus florida</i>	Dogwood
Sapindales	Aceraceae	<i>Acer saccharum</i>	Sugar maple
		<i>Acer rubrum</i>	Red maple
		<i>Acer negundo</i>	Box elder
	Anacardiaceae	<i>Mangifera indica</i>	Mango
Lamiales	Lamiaceae	<i>Salvia azurea</i>	Sage
Plantaginales	Plantaginaceae	<i>Plantago lanceolata</i>	English plantain
Scrophulariales	Oleaceae	<i>Fraxinus americana</i>	White ash
		<i>Ligustrum vulpura</i>	Privet
Asterales	Asteraceae	<i>Solidago</i> sp	Goldenrod
		<i>Erigeron philadelphicus</i>	Daisy fleabane
	Heliantheae	<i>Ambrosia artemisiifolia</i>	Short ragweed
		<i>Ambrosia trifida</i>	Giant ragweed
		<i>Cosmos bipinnatus</i>	Cosmos
		<i>Xanthium strumarium</i>	Cocklebur
	Anthemideae	<i>Chrysanthemum</i> sp	Chrysanthemum
		<i>Iva frutescens</i>	Marsh elder
Dipsacales	Caprifoliaceae	<i>Sambucus canadensis</i>	Elder

The amphiphilous plants that are not dependent on wind pollination are presented in boldface type.

TABLE III. The individual and cumulative prevalence of ≥ 10 mm diameter flare reactors to allergenic pollen extracts*

Quartile	No. of allergens	Allergenic pollen extracts	% Range of reactor prevalence
First	7	Red fescue, ryegrass, timothy, redtop, orchard, sweet vernal, short ragweed	27.6-19.1
Second	13	Giant ragweed, bluegrass, mesquite , Johnson, hickory, birch, corn , Burmuda, white oak, pigweed, red clover , beefwood, cosmos	18.7-11.1
Third	18	Sage, tag alder, beech, black oak, pecan, sweet gum, post oak, goldenrod , cocklebur, live oak, plantain, ash, red oak, sycamore, mulberry, lamb's-quarter, daisy fleabane , sheep sorrell	11.1-8.2
Fourth	30	European palm , rose, mimosa , box elder, red maple , sugar maple, elm, marsh elder, chrysanthemum , pussy willow , date palm, dogwood, aspen, acacia , black walnut, summer cypress , cedar, longleaf pine, poplar, alfalfa , privet, peach , elder , hibiscus, eucalyptus , shortleaf pine, mango , melaleuca , scrub pine, loblolly pine	8.0-2.2

*Skin tests were done with 68 allergenic extracts. Reactions ≥ 10 mm in diameter were detected in 325 (59%) of 550 patients. The 23 amphiphilous plants that are not dependent on wind pollination are presented in boldface type; mesquite, red clover, alfalfa, acacia, and melaleuca are zoophilous, and the remaining amphiphilous plants are entomophilous. Precise relative prevalences are not listed because the subjects were sampled from the southeast and may not apply to other regions of the United States.

years) patients who were tested during the interval between November 1974 and July 1981.

Evaluation of observations

Counts of the number and size of reactions to the pollens and related comparisons were made with FORTRAN (Microsoft, Redmond, Wash.) and a microcomputer. Statgraphics (STSC, Inc., Rockville, Md.) was used for the Kolmogorov-Smirnov tests to compare the distribution of the observed reactions with hypothetical distributions having the same mean and standard deviation and to obtain correlation and regression relationships between the size of reactions elicited by closely related allergenic extracts. Calculated odds ratios were used to estimate relative risk.

RESULTS

The identification of a positive skin test reaction is important. The minimum requirement for a positive test is a reaction area that is twofold or greater than the reaction to an appropriate negative control. The negative control can be diluent, saline, or even an allergen to which the subject is not sensitive. The

minimum requirement for a negative allergen skin test is the subject's positive reaction to an appropriate control. Skin tests with selected concentrations of methacholine were used as a positive control and permitted exclusion of patients with impaired cutaneous reactivity. The estimates of methacholine responsiveness and sensitivity in 434 patients were compared with previously reported observations⁸ in 84 healthy young subjects. This revealed that the allergic patients required a 1.9-fold greater methacholine concentration ($p \leq 0.001$) to elicit flare reactions equivalent in size ($p \leq 0.918$) to flare reactions detected in the healthy young subjects. Differences between skin test reactions to the saline control in patients and healthy subjects were small and not significant ($p \leq 0.546$). This comparison suggested that allergic patients may be less sensitive to the pharmacologic agonist than healthy young subjects. The contributions of patient age, sex, and disease to the complexity of this interesting finding are being evaluated and formulated into a separate article. Because of the exclusion of patients with suppression of methacholine reactivity and der-

TABLE IV. Informative allergenic pollen extracts in patients*

Allergenic pollen extract	No. of subjects	% Of subjects	Cumulative %
Red fescue	152	46.7	46.7
Mesquite	47	14.5	61.2
Short ragweed	32	9.8	71.0
Red clover	19	5.8	76.9
Timothy	13	4.0	80.9
Beech	10	3.1	84.0
Hickory	5	1.5	85.5
Sweet vernal	5	1.5	87.0
European palm	3	0.9	88.0
White oak	3	0.9	88.9
Privet	3	0.9	89.8
Melaleuca	2	0.6	90.4

*Tests with 24 additional allergens were needed to identify the remaining 10% of the 325 patients who had ≥ 10 mm flare-reaction diameters. Amphiphilous plants are presented in boldface type.

matographism, the observations in patients summarized in this article met the minimum basic requirements for positive skin tests.

The choice of summary statistics to describe the flare reactions elicited by pollen allergens is important. Cutaneous reactions (≥ 2 mm in diameter) were detected in 24396 (67%) of the tests. Only nine (1.6%) of the 550 patients failed to exhibit one or more detectable ≥ 2 mm reactions to any allergenic extract. The size of the skin test reactions to all 68 pollen allergens is summarized in Fig. 1. Evaluation of the size distribution of all skin test reactions with the Kolmogorov-Smirnov test revealed significant differences ($p \leq 0.0001$) from normal and log-normal distributions having the same means and standard deviations. The cumulative distribution (not presented) revealed that 22.4% of the skin test reactions were ≥ 5 mm in diameter, 10.4% of the reactions were ≥ 10 mm in diameter, 3.4% were ≥ 20 mm in diameter, and 1.3% were ≥ 30 mm in diameter.

The use of pooled reaction diameters to all 68 allergens could obscure a normal distribution of flare reactions to a single allergen. The distribution of reactions to individual allergens was also evaluated. The cumulative distributions of the size of skin test reactions produced by six representative pairs of allergenic extracts are summarized in Fig. 2. The paired-pollen extracts were selected from taxonomically closely related plants belonging to the same plant families. The distribution of skin test reactions to the individual pollen extracts also differed significantly from normal and log-normal distributions ($p \leq 0.01$). When the cumulative distribution of the size of the skin test reaction diameters of individual allergens was

plotted on a logarithmic scale, the distribution exhibited an interesting inflection in the size of reactions larger than 5 mm. Differences in the prevalence of reactions to fescue and ryegrass-pollen, giant and short ragweed-pollen, and mesquite-pollen extracts were detected beyond this inflection and appeared maximum with ≥ 10 mm diameter reactions. In contrast, considerable overlap was detected in the prevalence of reactions to the remaining seven pollen extracts in Fig. 2. This finding suggests that the distributions of skin test reaction diameters to the less reactive pollen extracts were equivalent.

A positive allergen skin test is also interpreted as evidence of a specific immune reaction to the allergenic extract. This interpretation means that the specificity of the skin test reaction is an important attribute of a positive reaction. The relationship between the detection of specific reactivity and the skin test reaction size is summarized in Fig. 3 with the same six pairs of closely related pollen extracts illustrated in Fig. 2. The degree of similarity between the compared skin test reactions is summarized as a correlation coefficient (r) and a plotted least-squares regression line. The departure of the compared reaction diameters in each patient from the regression line provides a graphic description of those skin test reactions that are specific for one or the other allergenic extract. Although exceptions exist (Fig. 3), specific reactions are difficult to detect with small (2 to 5 mm) skin test reaction diameters. A larger number of specific reactions can be detected with 6 to 9 mm flare-reaction diameters. The detection of specific skin test reactions is least ambiguous with ≥ 10 mm diameter flare reactions. This last conservative choice was selected as

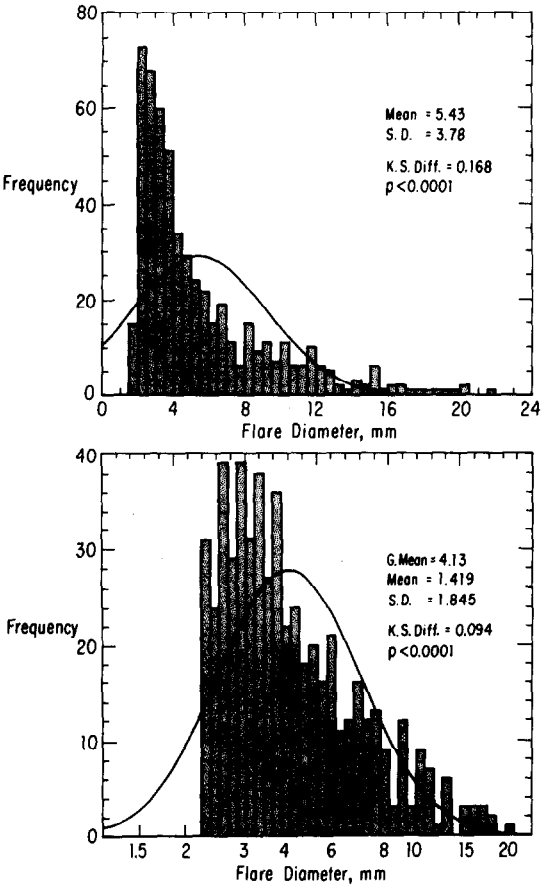


FIG. 1. The skin test reaction diameters elicited by epicutaneous (scratch) tests with allergenic pollen extracts are neither normally (**upper panel**) nor log-normally (**lower panel**) distributed. This graph describes the distribution of 24,396 skin test reactions elicited with 68 different allergenic pollen extracts in 550 patients. A Kolmogorov-Smirnov test was used to compare the observed distribution with a hypothetical normal distribution having the same mean and standard deviation. Highly significant differences were detected.

an arbitrary threshold for a positive skin test reaction.

The relative prevalence of ≥ 10 mm skin test flare-reaction diameters elicited with the 68 allergenic extracts is presented in Table III. Flare-reaction diameters of ≥ 10 mm were elicited in 325 (59%) of the 550 patients. The plant sources of the pollens from the most to the least reactive pollen extract are grouped and listed in Table III. The allergenic pollen extracts are also grouped with respect to the least number of extracts that are needed to detect cumulative quartiles of all observed positive reactions. The first column lists the successive quartiles. The second column lists the number of pollens within each quartile. The third column lists the common names of the pollen extracts in the quartile. The final column summarizes the range of the percent prevalence of reactions to the allergenic

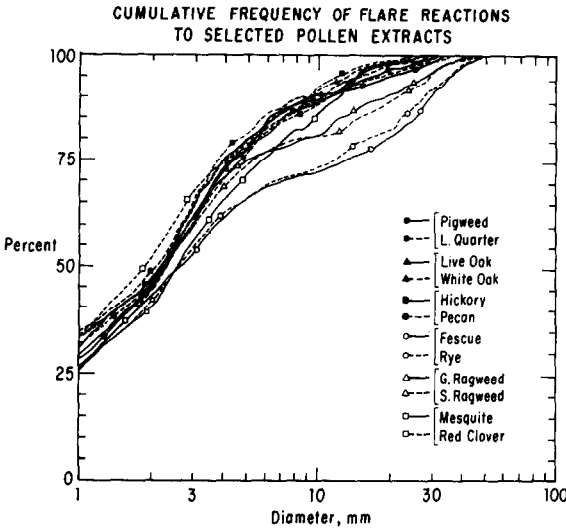


FIG. 2. Cumulative distribution of skin test flare reactions to selected pollen extracts is illustrated as the percent (abscissa) equal to or less than the reaction diameter (ordinate). Cumulative distributions were not linear even when a log scale was used to plot the size of the reaction diameters. The graph describes observations made with six selected pairs of allergenic pollen extracts. The paired pollens depicted in the *insert* belong to the same plant family. Note the inflection in the size distribution of the skin test reactions at 4 to 5 mm in diameter and the detectable differences in the prevalence of large (≥ 10 mm in diameter) reactions to fescue and rye-grasses, giant and short ragweed, and mesquite. The distribution of reaction diameters elicited with the allergenic extracts from the tree pollens and from pigweed and lamb's-quarter were remarkably similar.

extracts within the quartile. For example, as presented in Table III, seven anemophilous (wind borne) allergenic extracts accounted for a quarter of the positive flare reactions. The number and prevalence (percent) of subjects with positive reactions elicited with the seven extracts varied from 152 (27.6%) for red fescue grass to 105 (19.1%) for short ragweed. Six of the allergenic extracts were from members of the grass Poaceae family. Reactions to short ragweed skin test antigen, the single member of the Asteraceae family in this highly reactive group, were substantially less prevalent than reactions to the grass-pollen allergenic extracts.

The thirteen additional allergenic extracts that accounted for the second quartile of large reactions are also summarized in Table III. Four amphiphilous pollens contributed to the second quartile of reactions. Mesquite and red clover are zoophilous (animal borne) and corn and cosmos are entomophilous (insect borne) pollens. The number of subjects and prevalence (percent) of reactions to these 13 extracts varied from 103 (18.7%) for giant ragweed to 61 (11.1%) for cosmos.

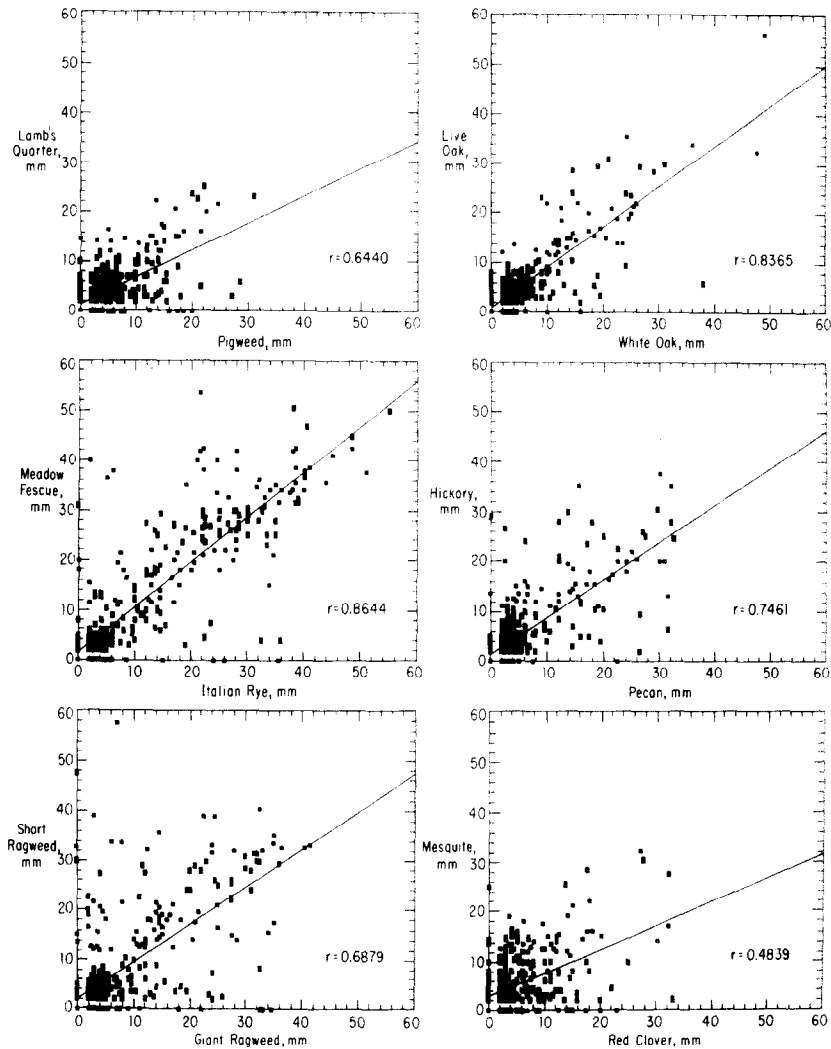


FIG. 3. Summarized in the graph are the relatedness and specificity of the skin test flare reactions elicited with the same six paired closely related allergenic extracts illustrated in Fig. 2. Paired reactions provide a specificity control for one another. *Each panel* describes the relatedness of the compared reactions as a correlation coefficient (r) and a least-squares regression line. The departure of each point from the line describes the specificity of the compared reactions within a single subject. The detection of specific reactions was least ambiguous with skin test flare-reaction diameters ≥ 10 mm.

This diverse group of antigens includes allergenic pollen extracts from eight plant families and includes the two families responsible for the first quartile of prevalent reactions. Collectively, half the positive skin test reactions were detected with 20 allergenic pollens from eight plant families.

An additional 18 allergenic extracts were necessary to elicit the third quartile of positive flare reactions. The third quartile of reactions required pollen extracts from eight additional plant families. The number of subjects and prevalence (percent) of reactions varied from 61 (11.1%) for sage to 45 (8.2%) for sheep sorrell. Two entomophilous pollens, goldenrod and daisy fleabane, were detected among the 18 allergens.

Skin tests with 38 allergenic pollen extracts from plants belonging to 16 families were needed to detect three quarters of the positive skin test reactions. Skin tests with 30 additional allergenic pollen extracts belonging to 12 additional plant families were needed to detect the last quartile of large reactions, as presented in Table III. The number of subjects and prevalence (percent) of reactions varied from 44 (8.0%) for European palm to 12 (2.2%) for loblolly pine. Sixteen of the 30 pollens in this last quartile are non-anemophilous. Acacia, alfalfa, and melaleuca are zoophilous, and the 13 remaining nonanemophilous pollens are entomophilous.

The description of observations in Table III pro-

vides one perspective of the relative importance of the generally available allergenic pollen extracts. This description does not identify the least number of allergenic pollen extracts needed to detect subjects with a single positive reaction to any pollen extract, such as might be useful in population-based studies of the incidence of allergen reactors. Large reactions to closely related pollen allergens often occur in patients. Examples of this association are illustrated in Fig. 3 and are considered presumptive evidence of crossed reactions to shared epitopes among components of the compared extracts. The observations summarized in Table IV ignores the tendency of allergic subjects to exhibit multiple large reactions and identifies only those extracts that were most likely to detect the 325 subjects who exhibited one or more positive reactions (≥ 10 mm flare) to any allergenic pollen extract. The description of observations presented in Table IV was prepared by counting the subjects who exhibited positive reactions to each allergenic extract and deleting the most reactive allergen and the subjects detected with this allergen from subsequent counts and selection of the next most informative allergenic extract. The order of importance of the 35 allergenic extracts that identified all 325 patients with reactions ≥ 10 mm in diameter is ranked in Table IV.

An allergenic extract of red fescue grass, as illustrated in Table IV, identified 152 (46.7%) of the 325 subjects. An allergenic extract of mesquite pollen identified 47 (14.5%) of the remaining 173 subjects and was clearly more informative than an allergenic extract of short ragweed, which identified 32 subjects (9.8%) among the remaining 126 patients. Allergenic extracts of red clover, timothy grass, and beech-tree pollen identified, respectively, 19 (5.8%), 13 (4.0%), and 10 (3.2%) subjects. Interestingly, mesquite, red clover and melaleuca are zoophilous (animal)-pollinated plants and European palm is an insect-pollinated plant. Collectively, these six allergenic extracts accounted for 84% of the 325 subjects with positive (≥ 10 mm in diameter) flare reactions. An additional 29 pollen extracts were necessary to identify the remaining skin test-positive subjects. The allergenic pollen extracts described in Table IV provide the largest amount of independent information about the prevalence of pollen hypersensitivity among the subjects.

DISCUSSION

Curiosity about several problems led to these studies. First, much of our use and understanding of the allergen skin test is based on intradermal (intracutaneous) methods. Scratch (epicutaneous) and puncture or prick (percutaneous) allergen tests are generally

considered comparable and substantially different from intradermal tests.^{9, 10} Epicutaneous and percutaneous tests are done with relatively high allergen concentrations in which device and potency-related differences are minimal, whereas relatively low allergen concentrations are used for intracutaneous tests. The only direct experimental comparisons of epicutaneous and intracutaneous tests with appropriate concentrations of the same allergens suggest that important differences can be detected.^{11, 12} This uncertainty obliges the specialist to make intuitive interpretations of epicutaneous and percutaneous reactions. The choice of allergenic pollen extracts for tests in patients is a second problem. Current knowledge of potentially important skin test allergens stems from studies of the prevalence of anemophilous (wind borne) pollens.¹³ Recent evidence suggests that the allergenic components of pollens are eluted rapidly¹⁴ and could be extracted by natural water into respirable atmospheric dusts.¹⁵⁻¹⁷ The possibility exists that the amphiphilous pollen allergens used in these tests could contribute to the occurrence of allergic disease. The criteria for interpreting the epicutaneous test reactions are a third problem. Much of our current knowledge about pollen hypersensitivity is based on the scaled (1 to 4+) intracutaneous reactions¹⁷ elicited with appropriate dilutions of pollen extracts. This arbitrary but useful descriptive scale differs from that used in population-based studies.¹⁻² A need exists to reconcile the information available from population studies with observations in patients.

These concerns led to evaluation of the large number of skin tests in the patients summarized in this article. Several general conclusions are possible. First, the distribution of reaction sizes summarized in Figs. 1 and 2 indicate that parametric descriptions may not provide an adequate summary of differences in epicutaneous flare reactions. The plot of measured skin test reaction diameters in Fig. 2 reveals three components. In addition to the negative tests and the first portion of each curve, a third component appears composed of reactions > 5 mm and is approximately linear for each allergen. This suggests that skin test reaction diameters in highly sensitive subjects could appear normally distributed. Parametric statistical comparisons may be valid only in selected exquisitely sensitive subjects. In randomly sampled subject groups, such as epidemiologic surveys, nonparametric statistical methods provide the more reliable basis for comparisons of skin test reactions to one concentration of an allergen.

Second, the observations summarized in Figs. 2 and 3 indicate that a ≥ 10 mm flare reaction unambiguously identifies those specific reactions that differ from ap-

TABLE V. A comparison of the prevalence of patient allergen reactors with the incidence of skin test reactors in the U.S. population

Allergenic pollen extract	% (p ₁) U.S. population (reference 2)	% (p ₂) Allergic patients (this study)	Estimated relative risk of allergy*
Rye grass	10.2	28.7	3.5
Ragweed	10.1	20.5	2.3
Oak	4.7	13.6	3.2
Bermuda	4.4	13.4	3.4

* $(p_2/(1-p_2))/(p_1/(1-p_1))$ where p_1 and p_2 are the decimal fraction expression of, respectively, the incidence of positive skin test reactions in the U.S. population and the prevalence of positive skin test reactions in patients. Differences in the compared odds ratio caused by withdrawal of the patient sample from the U.S. population are small and were not included in the calculations.

appropriate closely related specificity controls. This suggests that a ≥ 10 mm reaction diameter does provide a reliable, but conservative, basis for identifying a positive skin test reaction. Interestingly, this arbitrary choice is very close to the threshold selected in prior population-based epidemiologic studies with percutaneous allergen skin tests.^{1,2} This 10 mm flare reaction also agrees with conventional wisdom about the size of a 2+ skin test reaction.¹⁸ This test criterion appears especially useful in patients who are subjected to epicutaneous and percutaneous tests with a single allergen concentration. A ≥ 10 mm reaction diameter does provide a useful conservative criteria for a positive reaction in epidemiologic studies. In epidemiologic studies of the prevalence of subjects who react to allergenic extracts in the southeastern region of the United States, the 35 pollen extracts listed in Table IV would have identified all the ≥ 10 mm flare reactions detected with the 68 allergenic extracts. Finally, cutaneous hypersensitivity to grass-pollen allergens was more prevalent in these patients than reactions to weed- and tree-pollen allergens. In addition to the evidence presented in this article (Tables III and IV), this interesting conclusion is supported by the diversity and widespread distribution of grass species,⁶ their pollination throughout the growing season, and the wide-spread distribution of airborne grass pollens¹³ in the United States. Moreover, a pathogenic role of allergenic dusts from naturally eluted¹⁴⁻¹⁷ entomophilous and zoophilous pollens can not be excluded in patients with allergic disease.

The clinical use of these observations is also worth comment. Anecdotal reports suggest that at least two studies of pollen aerobiology have been done in North Carolina. The outcome of these studies are not available in the literature. The reactions listed in Table III are generally consistent with aerobiology studies from elsewhere in the southeast.¹¹ Regional differences in the prevalence of flora,⁶ wind-borne pollens,¹³

and cutaneous reactions to allergenic pollens² do exist and suggest that the relative prevalence of reactions listed in Table III may be limited to patients from the southeastern United States. The southeast has a prolonged warm growing season, diverse prolific flora, abundant rainfall, and high ambient humidity. Pollen-sensitive patients from this region often have perennial allergic symptoms with short periods of remission during the winter. A much needed quantitative appraisal is summarized in Table III of the relative prevalence of reactions to pollens in the southeastern United States and can be used to guide the selection of appropriate allergens for skin tests. The 20 allergenic extracts that elicit the first and second quartiles of reactions (Table III) identify the most important pollen allergens in the southeast. Although the 18 and 30 additional allergenic extracts of plant pollens, respectively, needed to elicit the third and fourth quartiles of flare reactions may have less clinical use, the relative reactor prevalence to these allergens was appreciable. The possibility of allergy to these allergens cannot be ignored in selected patients. When this information is used with a knowledge of the relative local abundance of plants and pollens, the number of skin tests needed for the initial evaluation of patients can be reduced¹⁹ with some logical expectation of the number of important allergens that might not be identified. These findings and similar studies of the prevalence of pollen reactors in other parts of the United States can be used to contain the health care costs of the allergic patients.

The expected similarity of the epicutaneous and percutaneous test methods^{9,10} appears confirmed by the independent selection of nearly identical thresholds for positive reactors in this and prior epidemiologic studies.² This suggests the prevalence of patient reactors to allergenic extracts of rye-grass, ragweed, oak, and Bermuda-grass pollens in this study can be compared to the estimated incidence of comparable

reactors in the U.S. population.² The estimated incidence of >10 mm diameter flare reactors in the U.S. population is compared with the relative prevalence of ≥ 10 mm diameter flare reactions in this patient sample in Table V. Estimates of the relative risk of allergic disease associated with a positive skin test are also presented in Table V. The twofold to fourfold risk associated with a positive skin test is small in relation to the level of confidence needed to establish a diagnosis and implement appropriate therapy. The small relative risk associated with detection of large allergen-induced skin test reactions is consistent with contemporary cautions about the use of skin tests in the remote practice of allergy.²⁰ The allergen skin test can be used to corroborate the clinical evaluation of the patient. A positive allergen skin test alone cannot be used to establish the diagnosis of an allergic disease.

Finally, the arbitrary selection of a ≥ 10 mm flare-reaction diameter as a threshold positive reaction for comparisons of relative prevalence does not imply that smaller skin test reactions are not meaningful. It is clearly indicated in Fig. 3 that a small number of paired positive and negative reactions to closely related allergens could be identified in the subject sample. Judgments about the meaning of a skin test reaction must be based on clinical evaluation of the patient, the exposure history, and an appraisal of other allergen reactions in the particular patient.

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