

Use of standardized and conventional allergen extracts in prick skin testing

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This study examined whether commercially available conventional and standardized allergen extracts differ enough in potency to affect routine prick skin test results. Extracts of white oak, timothy, Bermuda, Russian thistle, short ragweed, sagebrush, Alternaria, and cat dander were examined in allergic patients and in nonatopic subjects with no personal or family history of asthma, rhinitis, or eczema. Conventional nonstandardized extracts (1:10 or 1:20 wt/vol) from two sources were compared with three concentrations (100,000, 10,000, and 1000 AU/ml) of a single standardized extract. Preparations were compared in the allergic patients with computerized planimetry, and in all patients and subjects with a conventional skin test grading system. Skin test area for the conventional extracts generally fell between the 10,000 and 100,000 AU/ml concentrations of the standardized extract. Skin test reactivity to at least one allergen extract occurred in 31% of the nonatopic subjects; there was no difference between the number of 3+ and 4+ reactions for conventional and standardized extracts. Results indicate that standardized and conventional extracts are frequently similar, but are not directly interchangeable. (J ALLERGY CLIN IMMUNOL 1992;89:658-66.)

Key words: Allergen extracts, skin testing, atopy, standardization

During the past 20 years, a number of practical methods have been developed to minimize the variation in allergen content and potency that has been found with conventional allergen extracts.^{1, 2} Currently, a variety of standardized extracts is commercially available, and these extracts are being used by an increasing number of practicing allergists. Initial clinical experience with these extracts has led to the impression that the standardized preparations are more potent than conventionally made extracts, although this finding has not been demonstrated in a clinical

study. If standardized extracts were more potent, larger than usual skin test reactions would be expected in allergic patients as well as a higher prevalence of positive skin tests in an asymptomatic nonatopic population.³ This study was performed to determine whether standardized allergen extracts produce larger skin test reactions than conventional extracts when these extracts are used clinically for prick skin testing and whether there is a difference in the number of positive skin tests obtained in nonatopic subjects.

MATERIAL AND METHODS

Extracts

The inhalant allergen extracts used in this study (Table I) were white oak (*Quercus alba*), timothy (*Phleum pratense*), Bermuda (*Cynodon dactylon*), Russian thistle (*Salsola pestifer*), short ragweed (*Ambrosia artemisiifolia*), sagebrush (*Ambrosia ludoviciana*), *Alternaria tenuis*, and cat dander (*Felis domesticus*). For each inhalant, two conventional extracts (1:10 or 1:20 wt/vol) were obtained from different manufacturers (Hollister-Stier Laboratories, Spokane, Wash., and Allergy Laboratories of Ohio, Columbus, Ohio). The conventional extracts were used as supplied by the manufacturer for prick skin testing in 50% glycerol/saline. These extracts were compared with three concentrations (100,000, 10,000, 1000 AU/ml) of corresponding standardized extracts obtained in lyophilized form from three sources (Pharmacia, Milford Conn.; Hollister-Stier Laboratories; and 3M Diagnostic Systems, Mountain View,

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The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of Defense. The protocol under which this study was conducted was approved by the Clinical Research and Human Use Committee of Fitzsimons Army Medical Center.

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TABLE I. Allergen extracts used*

Inhalant	Standardized	Conventional No. 1 (wt/vol)†	Conventional No. 2 (wt/vol)‡
White oak (<i>Quercus alba</i>)	Pharmacia Lyophilized	1:20 Exp Oct. 88	1:20 Exp Dec. 90 48,000 PNU/ml
Timothy (<i>Phleum pratense</i>)	Hollister-Stier Lyophilized	1:20 Exp Aug. 88	1:20 Exp Dec. 90 44,000 PNU/ml
Bermuda (<i>Cynodon dactylon</i>)	Pharmacia Lyophilized	1:20 Exp Aug. 88	1:20 Exp Dec. 90 40,000 PNU/ml
Russian thistle (<i>Salsola pestifer</i>)	Hollister-Stier Lyophilized	1:20 Exp Oct. 88	1:20 Exp Dec. 90 41,000 PNU/ml
Short ragweed (<i>Ambrosia artemisiifolia</i>)	Hollister-Stier Lyophilized	1:20 Exp Aug. 88, Ag E: 63 U/ml§	1:20 Exp Dec. 90 Ag E: 278 U/ml§ 70,000 PNU/ml
Sagebrush (<i>Artemisia tridentata</i>)	3M Lyophilized	1:20 Exp Oct. 88	1:20 Exp Dec. 90 49,000 PNU/ml
<i>Alternaria tenuis</i>	3M Lyophilized	1:10 Exp July 88	1:20 Exp Dec. 90 14,000 PNU/ml
Cat dander (<i>Felis domesticus</i>)	ALK America Lyophilized	1:10 Exp June 88	1:20 Exp Dec. 90 29,000 PNU/ml

Exp, Expiration date.

*The standardized extracts were reconstituted at concentrations of 100,000, 10,000, and 1000 AU/ml in 50% glycerin/phosphate-buffered saline, pH 8.0. The conventional extracts were used as supplied by the manufacturer in 50% glycerosaline for prick skin testing.

†Obtained from Hollister-Stier Laboratories; protein nitrogen unit content not available.

‡Obtained from Allergy Laboratories of Ohio.

§Ag E (antigen E) content of short ragweed (*Amb a 1*).

||Milford, Conn.

Calif.). The standardized extracts were labeled by the manufacturers on the basis of comparison to 1:10 wt/vol reference preparations. The standardized extracts were reconstituted and diluted in 50% glycerin/phosphate-buffered saline with the pH adjusted to 8. Extracts were obtained from the freshest lots commercially available. The glycerol/saline was used as a negative control, and histamine phosphate solution (histamine base 1.8 mg/ml) was used as a positive control.

Patients and subjects

All patients and subjects were required to be between the ages of 18 and 70 years. Allergic patients were recruited from the allergy clinic population on the basis of previous 3 to 4+ prick skin test reactivity (as defined by criteria below) to one or more of the inhalants tested. The nonatopic subjects were not recruited from the allergy clinic. Prospective subjects were asked to complete a questionnaire concerning personal and family history of asthma, rhinitis, eczema, or other rashes and antiallergic drug use (Table II), patterned after a similar questionnaire used in a previous

study.⁴ Subjects were excluded for any "yes" answer and were thus required to have a negative personal as well as family history of asthma, rhinitis, and eczema. Subjects with a negative personal and antecedent family history, but with atopic offspring, were excluded even if an atopic spouse could account for the atopic children. The decision to exclude subjects was made before skin testing.

Skin testing and measurements

The prick method of epicutaneous skin testing was used for all skin tests. Skin tests were performed in singleton. A drop of extract was placed on the back, and the skin beneath it was pricked with a sterile straight needle (Hollister-Stier Laboratories) entering the epidermis at a 45 degree angle and lifted straight up. The needle was wiped with an alcohol sponge after each prick, and results were read after 15 minutes. Positive skin tests were outlined on the skin with a fine-tipped felt pen and transferred to paper with transparent tape.

Skin test response in both the allergic patients and non-atopic subjects was graded semiquantitatively on a scale of

TABLE II. Medical history questionnaire

Personal history
Have you ever had
Wheezing with colds
Asthma
Eczema
Skin rash or wheezing with food
Constant nasal drainage or obstruction
Frequent nasal itching or sneezing
Intolerance to pets because of allergy
Seasonal itching of the eyes or nose, sneezing, watery nasal discharge
Family history
Did your mother, father, brother, sister, or children ever have any of the above symptoms?
Medications*
Do you regularly take
Antihistamines (CTM, Actifed, Dimetapp, Drixoral, Sinutab, Allerest, Dristan, A.R.M., etc.)
Decongestants (Entex, Sudafed)
Terbutaline
Theophylline

*CTM, Schering-Plough, Kenilworth, N.J.; *Actifed*, Burroughs Wellcome Co., Research Triangle Park, N.C.; *Dimetapp*, A.H. Robins Co., Inc., Richmond, Va.; *Drixoral*, Schering Corp., Kenilworth, N.J.; *Sinutab*, Parke-Davis, Morris Plains, N.J.; *Allerest*, Pharmacrast Div., Rochester, N.Y.; *Dristan*, Whitehall Laboratories, New York, N.Y.; *A.R.M.*, SmithKline Consumer Products, Philadelphia, Pa.; *Entex*, Norwich Eaton Pharmaceuticals, Inc., Norwich, N.J.; *Sudafed*, Burroughs Wellcome.

0 to 4+, as is recommended for clinical use,⁵ and as is the practice of this clinic. Skin tests of 1+ reactivity were defined as reactions with erythema but no wheal; 2+ skin test reactions were reactions with a wheal diameter of <3 mm; 3+ skin test reactions were reactions with no pseudopods and a wheal diameter of 3 mm or larger; 4+ skin test reactions were reactions with pseudopods and a wheal diameter of 3 mm or greater. Skin test results graded 3 to 4+ were considered positive.

Additionally, the areas of the wheal-and-flare reactions were measured by computer planimetry (Bioquant II, R & M Biometrics, Inc., Nashville, Tenn.) in the allergic patients, but not in the atopic subjects. The same investigator performed all skin tests and made all measurements.

Data analysis

Relative skin test reactivity was assessed by comparing mean wheal-and-flare areas for each of the three concentrations of standardized extract and for the two conventional extracts by repeated measures analysis of variance at the $\alpha = 0.05$ level. The Newman-Keuls' test was used for paired multiple comparisons; because of the large number of multiple comparisons, the $\alpha = 0.01$ level was considered significant. Measurements of wheal-and-flare area obtained in the allergic patients were examined separately. The number of positive and negative skin test results or the nonatopic subjects were compared by a chi-square analysis of a 2 by 5 contingency table at the $\alpha = 0.05$ level.

RESULTS

Twenty allergic patients were tested with each extract with the exception of white oak, cat, and *Alter-*

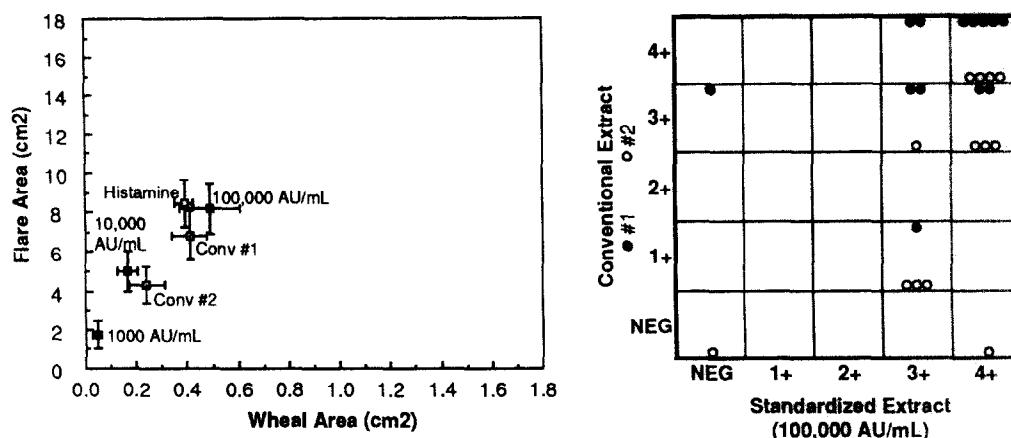
naria, for which 13, 17, and 13 patients were tested, respectively. Fewer than 20 subjects were tested with these extracts because skin test positivity to these extracts was found to be uncommon in the clinic population. Sixty-two subjects were recruited as possible nonatopic subjects, but 17 were eliminated before skin testing on the basis of questionnaire responses. Forty-five nonatopic subjects therefore underwent skin testing to all eight sets of extracts.

All skin testing was performed between the months of August 1987 and May 1988. Therefore, testing was completed before the expiration dates of the extracts, with the exception of short ragweed conventional extract No. 1. There was no difference in reactivity between this extract and the short ragweed conventional extract No. 2 (wheal-and-flare areas, *p*, not significant).

Comparisons of skin test reactivity of the extracts in the allergic patients are illustrated in Figs. 1 to 4. Histamine wheal-and-flare response was similar for all testing, with wheal areas ranging 0.30 to 0.42 cm² and flare areas in the range of 7.2 to 8.7 cm², with the exception of the patients tested with cat extracts in whom histamine produced a flare of 4.3 cm². The reason for this difference is not clear. All glycerol/saline control sites produced completely non-reactive responses.

For the nonatopic subjects, skin test areas were not measured because of the intent to evaluate prevalence of a positive reaction. All subjects demonstrated his-

A. White Oak



B. Timothy

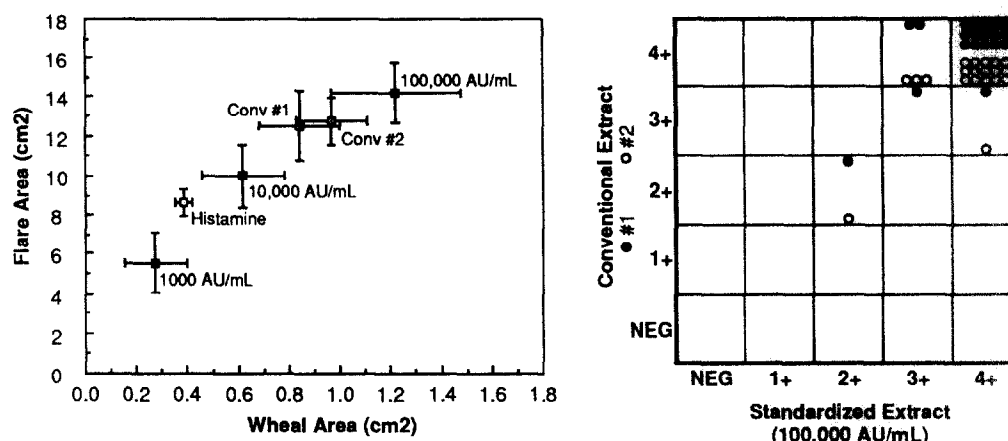


FIG. 1. A, Skin test results for white oak. B, For timothy. Boxes on the left demonstrate mean areas for wheal (*abscissa*) and flare (*ordinate*) in square centimeters for the two conventional extracts (Conv No. 1 and Conv No. 2), the three concentrations of the standardized extract (1000, 10,000, and 100,000 AU/ml) and the histamine control. Vertical bars represent SEM for flare area and horizontal bars are SEM for wheal area. Boxes on the right demonstrate correlation of results of two conventional extracts with results of 100,000 AU/ml standardized extract in terms of 0 to 4+ grades (see text for details).

tamine responses graded 3 to 4+, and all subjects had entirely nonreactive glycerol/saline controls.

The two conventional extracts produced similar results in seven of the eight extracts (p , not significant). White oak conventional extract No. 2 demonstrated smaller wheal-and-flare areas than extract No. 1 and standardized extract at both 100,000 and 10,000 AU/ml concentrations ($p < 0.1$). The conventional extracts otherwise consistently fell between 10,000 and 100,000 AU/ml in reactivity. In all systems, the 1000 AU/ml concentrations demonstrated significantly less reactivity than histamine ($p < 0.01$).

Wheal-and-flare areas were largest for the stan-

dardized 100,000 AU/ml concentration in seven of eight extracts but were only statistically different for Bermuda, sagebrush, and cat ($p < 0.01$). Both conventional *Alternaria* extracts elicited marginally greater skin test reactions than the standardized 100,000 AU/ml concentration (p , not significant). The three extracts producing the least frequently encountered skin test reactivity in the allergic patient population (white oak, *Alternaria*, and cat) also consistently produced smaller reactions than the other extracts.

Skin test reactivity was also graded on a conventional 0 to 4+ scale for the 100,000 AU/ml stan-

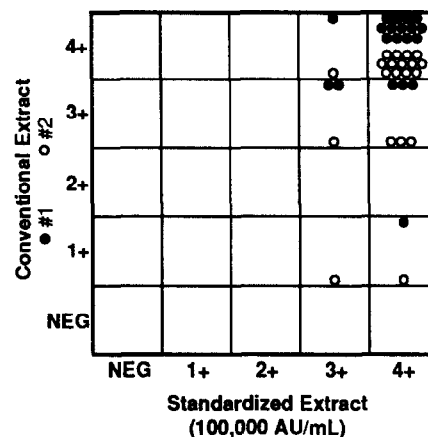
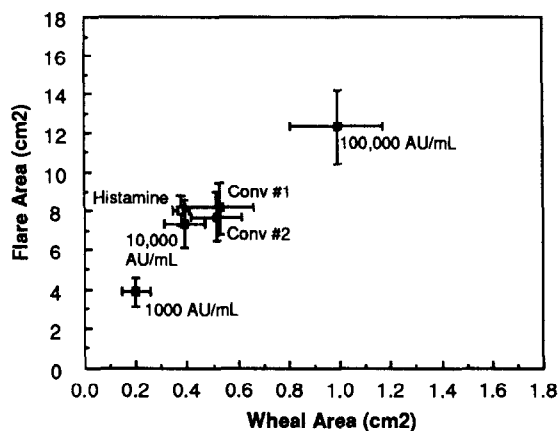
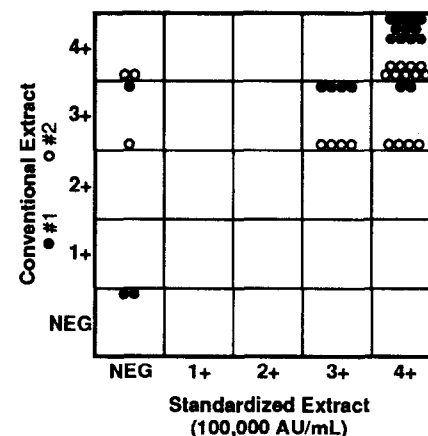
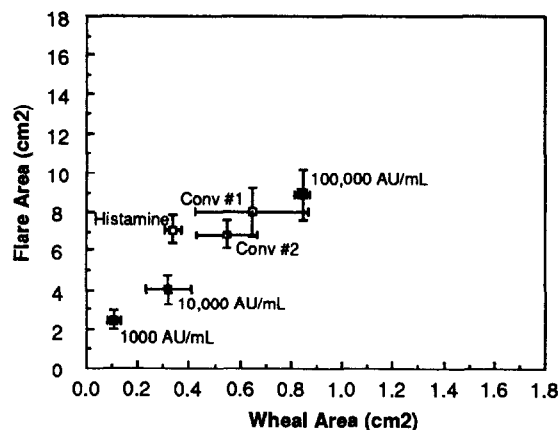
A. Bermuda**B. Russian Thistle**

FIG. 2. A, Skin test results for Bermuda. **B,** For Russian thistle. See legend to Fig. 1 for details.

dardized extract and each conventional extract in the allergic patients. Six of eight extracts produced essentially the same number of 3+ and 4+ reactions with standardized and conventional extracts (Figs. 1 to 4). For cat and white oak, the standardized extract produced more 3+ and 4+ reactions than did the conventional extracts. Three patients had 3 to 4+ reactivity to one or both conventional Russian thistle extracts but were negative to the standardized extract.

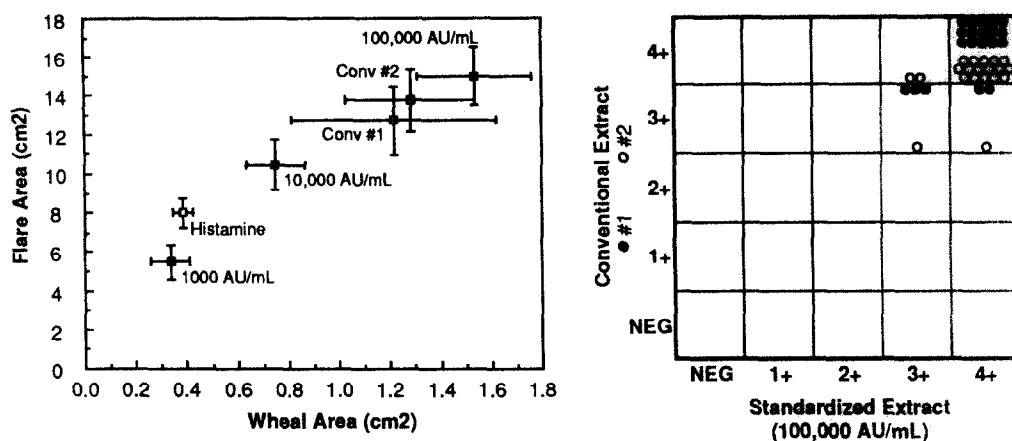
Skin test results for the nonatopic subjects are illustrated in Fig. 5 and in Table III. Fourteen of 45 subjects (31%) had a 3 to 4+ skin test to at least one extract. The number of positive tests ranged from 1 to 7. For each extract, there was no significant difference between the proportion of subjects reacting to the three dilutions of standardized extract or the two conventional preparations. Extracts, such as timothy and short ragweed, which produced larger skin test

areas in the allergic patients, tended to produce a larger number of 3 or 4+ reactions in the nonatopic subjects.

DISCUSSION

In the eight allergen extracts evaluated in this study, a concern that standardized extracts might produce markedly larger skin test reactions than conventionally labeled extracts was only partially justified. Although the full strength standardized extracts (100,000 AU/ml) generally elicited larger wheal-and-flare areas than the two conventional extracts, this difference achieved statistical significance only with Bermuda, sagebrush, cat dander, and one of the white oak conventional extracts, and might not have been noticed by the practitioner who uses a semiquantitative grading system. Even though the Bermuda, sagebrush, cat, and white oak standardized extracts produced larger

A. Short Ragweed



B. Sagebrush

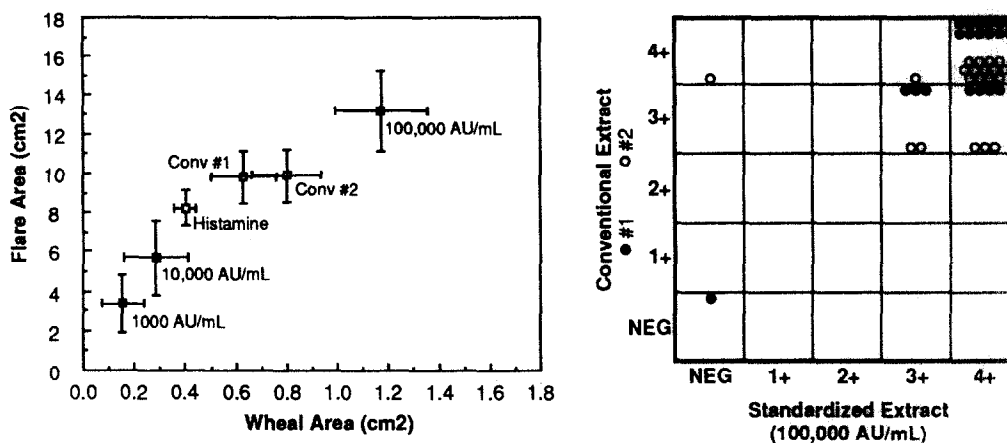


FIG. 3. A, Skin test results for short ragweed. B, For sagebrush. See legend to Fig. 1 for details.

wheal-and-flare reactions in allergic patients, there was not an increase in clinically irrelevant positive skin test results in the nonatopic subjects (Fig. 5).

The standardized extracts used in this study were labeled by the manufacturer with arbitrary unitage on the basis of a comparison to a reference extract originally supplied at a 1:10 wt/vol concentration. The smaller skin test results of the conventional extracts used in the present study could be related to the use of 1:20 wt/vol (rather than 1:10 wt/vol) extracts, but this relationship is unlikely because a twofold difference in potency would produce only a small change in wheal size.⁶ Indeed, examination of results from the two 1:10 wt/vol conventional extracts used in this study suggests that this difference did not have a major impact.

The 10,000 AU/ml dilution of standardized extracts offered no advantage over conventional extracts for

diagnostic prick testing. In fact, for four of eight extracts, this concentration produced reactions smaller than the reactions of histamine control.

Examination of the wheal-and-flare areas suggests that the standardized extracts might produce results in a higher skin test grading class. This finding occurred with Bermuda, white oak, and cat dander (Figs. 1, 2, and 4) but was not apparent with timothy, short ragweed, or sagebrush (Figs. 1 and 3). Notably, for *Alternaria* (Fig. 4), skin test results graded on a 0 to 4+ scale appeared to correlate less well, despite similar mean skin test areas. Three patients with 3 to 4+ reactivity to one or both conventional Russian thistle extracts were negative when they were tested with the 100,000 AU/ml standardized extract. These discrepancies could reflect variability in the skin testing procedure or differing content of minor allergens important for individual patients.

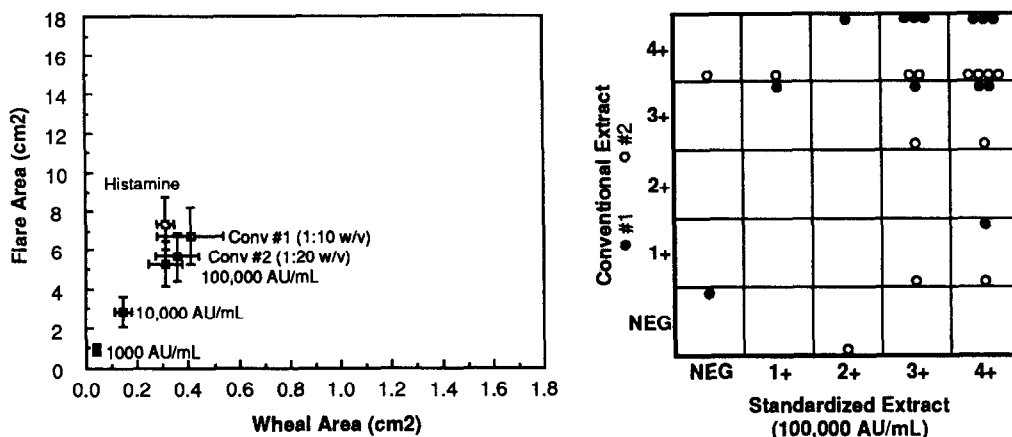
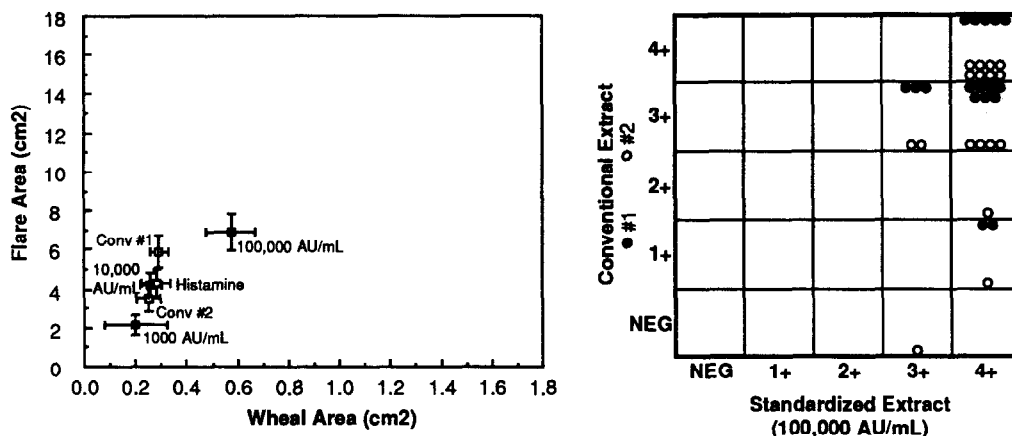
A. *Alternaria***B. Cat Dander**

FIG. 4. A, Skin test results for *Alternaria*. B, For cat dander. See legend to Fig. 1 for details.

Of the carefully selected nonatopic subjects, 31% had a positive skin test to one or more extracts. This number is higher than the 9% prevalence of positive skin tests, reported by Curran and Goldman in 1961⁴ and the 4% prevalence found by Pearson in 1937,⁷ but is comparable to results of a recent study of standardized (but not conventional) extracts by Adinoff et al.⁸ The subjects in these studies had both negative personal and family histories of atopic diseases. Studies in which subjects have no personal history, but a positive family history, of atopic disease,^{4, 8, 9} demonstrate from a 20% to a 50% prevalence of positive skin tests. The subjects in the present study were carefully screened for any history of symptoms that might be suggestive of atopic disease and for any family history of atopic disease, although it is conceivable that atopic individuals were not entirely excluded by

the questionnaire and interview. The reason for this higher prevalence of positive skin tests for both standardized and conventional extracts is unclear, but results emphasize the concept that the finding of a positive skin test (although it is demonstrative of allergic sensitization) does not alone signify the presence of disease.

In the allergen extracts examined in this study, it appears appropriate to use the 100,000 AU/ml concentration of standardized extracts for routine prick skin testing. The prevalence of clinically irrelevant positive results, as defined by reactions in nonatopic subjects, is not increased over the prevalence of that observed with conventional nonstandardized extracts. It should be emphasized that this study did not evaluate the question of lot-to-lot variability in allergen composition or potency and that results for the extracts

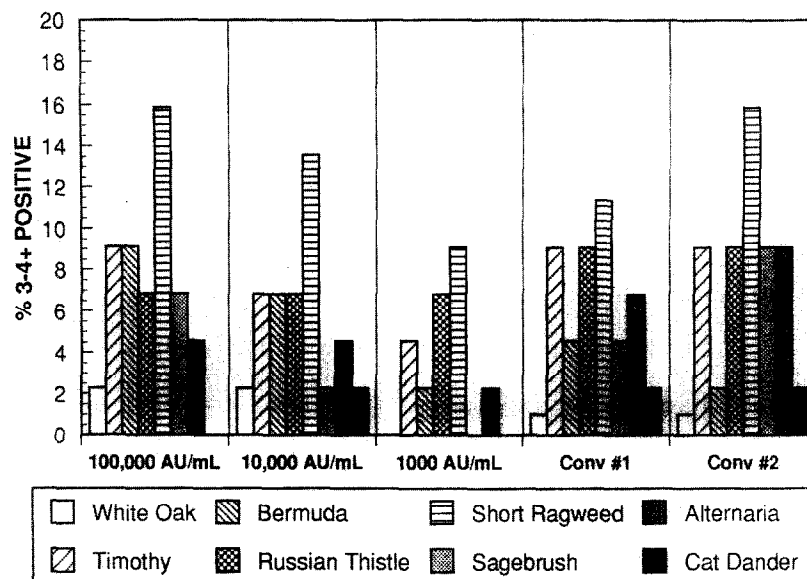


FIG. 5. Proportion of nonatopic subjects reacting to three concentrations of standardized extract (100,000, 10,000, and 1000 AU/ml) and the two commercial extracts (Conv No. 1 and Conv No. 2) for the eight extracts studied.

TABLE III. Results of testing of nonatopic subjects

Patient No.	Allergen extracts								Total*
	White oak	Timothy	Bermuda	Russian thistle	Short ragweed	Sage	Alternaria	Cat	
10	S,C1,C2	S,C1,C2	S	S,C1,C2	S,C1,C2	C2	C2		7
31		S,C1,C2	S	S,C1,C2	S,C1,C2	S,C1,C2			5
15				S	S	S,C2		S	4
24			S,C1	S,C1	S	S,C2			4
38				C1,C2	S,C1,C2	C1,C2			3
43		S,C1,C2		C2			S,C1,C2		3
9			S,C1,C2				C2		2
18					S,C2	S,C1,C2			2
40		S,C1,C2				S,C1,C2			2
4					S,C1,C2				1
6							S,C1,C2		1
11					S,C1,C2				1
19							S,C1		1
23								S,C1,C2	1
Total†	1(2.2%)	4(8.8%)	4(8.8%)	6(13.3%)	8(17.7%)	7(5.5%)	5(11.1%)	2(4.4%)	

S, Standardized extract; C1, conventional extract No. 1, C2, conventional extract No. 2.

Nonatopic subjects, 14/45 (31.1%), had 3 to 4+ reactions to one or more of the eight test extracts.

*Number of specific allergen extracts to which each subject reacted.

†Number (percentage) of nonatopic subjects with a positive reaction to this specific allergen extract.

used in this study may not be applicable to other extracts or lots of extracts. Additionally, results suggest that intensity of exposure to regional allergens may impact on relative skin test reactivity.

Although standardized and conventional extracts may, in some cases, be similar, they are not directly interchangeable. The clinician who wishes to use standardized extracts in practice may want to perform

comparative skin testing initially to compare the new standardized extracts to extracts with which he is familiar.

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Connective tissue-activating peptide-III and its derivative, neutrophil-activating peptide-2, release histamine from human basophils

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*Connective tissue-activating peptide-III (CTAP-III) is a 9 kd platelet α -granule-derived growth factor. It stimulates the synthesis of DNA, hyaluronic acid, glycosaminoglycans, and proteoglycan core protein in human fibroblasts. Human mononuclear cell-derived proteases have been previously demonstrated to digest the N-terminal 15 residues of CTAP-III (total, 85 residues) to produce neutrophil-activating peptide-2 (NAP-2). CTAP-III and NAP-2 belong to a class of proteins (platelet factor 4, interleukin-8/NAP-1, etc.) associated with inflammation and wound repair. In our efforts to purify human mononuclear cells and platelet-derived histamine-releasing factors, we had previously discovered that mixtures of CTAP-III and NAP-2 released histamine from human basophils. We have now developed simple protocols for the purification of CTAP-III and NAP-2, independently, from calcium ionophore (A23187)-stimulated platelet supernatants by affinity chromatography and have established their identity by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and N-terminal sequence analysis. Each of these released histamine between 2 and 10 μ g/ml, a range identical to that obtained with CTAP-III/NAP-2 mixtures that we reported earlier. Thus, our data suggest that CTAP-III and NAP-2 independently release histamine from human basophils in dose ranges similar to ranges required for fibroblast stimulation by each. (*J ALLERGY CLIN IMMUNOL* 1992;89:666-72.)*

Key words: Connective tissue, neutrophil, activating peptides, fibroblast growth factors, histamine release, basophils, protein purification

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