

23521

BALD CYPRESS TREE (*TAXODIUM DISTICHUM*) POLLEN, AN ALLERGEN

GERALD A. BUCHOLTZ, MD; RICHARD F. LOCKEY, MD; and
DEBORAH SERBOUSEK, BA

Fifty-seven nasal and bronchial provocation tests were performed with bald cypress pollen extract (BCE) on 41 subjects. A positive nasal response was elicited in 12 of 17 (71%) subjects with allergic rhinitis and positive BCE skin tests, and in none of 16 control subjects. A positive bronchial challenge was elicited in two of ten extrinsic asthmatics with positive BCE skin tests, and in none of 14 control subjects. BCE-specific IgE was demonstrated by RAST in seven of the 12 (59%) subjects with a positive nasal challenge and in one of the two with a positive bronchial challenge. BC pollen is an aeroallergen.

Introduction

THERE ARE THREE SPECIES in the genus *Taxodium* belonging to the family Taxodiaceae. The bald cypress, *Taxodium distichum* Rich., and the pond cypress, *Taxodium ascendens* Brogn., are found throughout the wetlands of the southeastern United States.¹ *Taxodium mucronatum* is found in Mexico. None of these species have been evaluated as a source of aeroallergens.² The Japanese cedar, *Cryptomeria japonica*, is a member of the Taxodiaceae family and considered an important aeroallergen in Japan.³ Yasueda et al have isolated and partially characterized a major aeroallergen "major basic protein" from the crude extract of Japanese cedar pollen.⁴ Three other investigators have studied the allergenicity of cypress tree pollen,⁵⁻⁷ however, these belong to the family Cupressaceae and not the family Taxodiaceae. Experimental evidence suggests a lack of cross-reactivity between these two families.⁸

Some taxonomists consider the bald cypress and pond cypress to be a variation of a single species. They are prolific producers of pollens which have an identical structure and are collectively counted as cypress pollen. The highest daily cypress pollen count, recorded at the University of South Florida, Tampa is 2,791 pollen grains per cubic meter of air sampled. This compares

to the highest pollen count of oak, 3,510 pollen grains per cubic meter of air sampled.⁹

The proper evaluation and treatment of patients with allergic rhinitis and extrinsic asthma depends on the selection of significant aeroallergens in the preparation of antigens to be used for *in vivo* and *in vitro* testing and immunotherapy. Although bald cypress pollen extract (BCE) is commercially available for skin testing and immunotherapy, the bald cypress pollen has not previously been evaluated as an aeroallergen.²

An aeroallergen-producing plant should fulfill Thommen's five postulates.¹⁰ The bald cypress tree is a suspected source of an aeroallergen because it fulfills four of Thommen's five postulates: (1) it is abundant and widely distributed in swamps and along rivers in the southeastern United States (Fig 1), (2) it produces wind-born pollen, (3) it produces large amounts of pollen from December through April, and (4) its pollen is buoyant (28 to 32 μ) (Fig 2).¹¹ This study provides evidence for the remaining postulate, ie, allergenicity.

Materials and Methods

Subjects

Forth-one volunteer subjects, 24 males and 17 females, ages 13 to 57 years, were selected from the outpatient allergy and immunology clinics of the James A. Haley Veterans Administration Hospital, the University of South Florida College of Medicine and from the private offices of several Tampa Bay area physicians. Excluded from study were subjects with cardiac disease, irreversible lung disease, pregnancy, or previous immunotherapy with BCE. All subjects gave written

From the Division of Allergy and Immunology, Department of Internal Medicine, University of South Florida College of Medicine and the James A. Haley Veterans Administration Hospital, Tampa, Florida.
Supported by a grant from the Medical Research Service of the Veterans Administration.

BALD CYPRESS TREE POLLEN — BUCHOLTZ ET AL



Figure 1. Distribution of *Taxodium* in the United States: bald cypress (*Taxodium distichum*) both shaded areas and Pond Cypress (*Taxodium ascendens*) in darkest shaded area. Adapted from Little E. Atlas of United States Trees, Volume 1 Conifers and Important Hardwoods. U.S. Department of Agriculture. Publication # 1146.

informed consent as approved by the University of South Florida Committee of Associates on Human Experimentation.

Medications

The following medications were withheld prior to skin testing: nonsteroidal anti-inflammatory drugs, 24 hours; antihistamines, 48 hours; and hydroxyzine HCl, 96 hours. The following medications were withheld prior to nasal challenges: decongestants, 24 hours; antihistamines and nasal glucocorticosteroids, 48 hours; hydroxyzine HCl, 96 hours; and nasal cromolyn, 4 weeks. The following medications were withheld prior to bronchial challenge: inhaled beta-2-agonists, alpha-adrenergic antagonists and anti-cholinergics, eight hours; oral beta-2-agonists and methylxanthines, 12 hours; non-steroidal anti-inflammatory agents, 24 hours; antihistamines, 48 hours; hydroxyzine HCl, 96 hours; and cromolyn, 4 weeks. No subjects had oral glucocorticosteroid within 2 weeks prior to nasal or bronchial challenge.

Bald Cypress Tree Pollen Extract

The commercially available 1:10 wt/vol aqueous BCE, in 0.4% phenol (supplied by Greer Laboratories, Lenoir, NC, 28645, lots #193-14-1A5, #193-14-1A6 and #193-14-1A10) were prepared from the same batch of BC pollen harvested March 1978 and stored at 4° C until used. All dilutions for skin and challenge testing

were made just before use with a diluent containing 0.4% phenol, 0.5% sodium chloride, and 0.275% sodium bicarbonate diluent (Coca's solution).

Skin Testing

Intradermal skin tests were performed with 0.02 mL 1:500 wt/vol BCE and read 20 minutes post-injection. Histamine 0.1 mg/mL was used as a positive control and the diluent as a negative control. The skin test was considered positive if the mean diameter of the wheal minus the mean diameter of the diluent control wheal was ≥ 8 mm.¹²

RAST

Blood was collected prior to skin testing and challenges and the serum stored at -70° C. Discs for the RAST procedure were prepared in our laboratory by coupling 20 mL of 1:100 wt/vol BCE lot #193-14-1A6 to 200 6-mm, cyanogen-bromide-activated paper discs supplied by Pharmacia, Upsala, Sweden.¹³ The RAST assay was done in duplicate by incubating the disc with 100 μ L of the subject's serum for 20 hours followed by addition 50 μ L of ¹²⁵I goat anti-human IgE and counts were made on a Packard gamma counter. RAST results were expressed as percent of total counts bound per minute (TCBM).

Nasal Challenge

Double-blind nasal challenges (NC) were performed on 33 subjects in two groups. *Group 1-NC* included 17 subjects with symptoms of allergic rhinitis during the BC pollinating season and positive intradermal skin tests with BCE. *Group 2-NC* was comprised of 16 subjects with negative intradermal skin tests with BCE (six with allergic rhinitis and positive prick skin test to two or more common local aeroallergens, four with vasomotor rhinitis and negative prick skin test to common local aeroallergens, and six normal subjects).

After baseline nasal examination, NCs were performed by randomizing the sequence of gentle dropping of 60 μ L of 1:1000 wt/vol of BCE on one inferior turbinate and 60 μ L of diluent on the other. The challenge was double-blind, neither the observer nor the patient having knowledge of which side received the extract. After 20 minutes, subjects were questioned for symptoms of allergic rhinitis (pruritus, sneezing, congestion), and the nasal mucosa was examined for changes (edema, rhinorrhea, palor, and mucus stringing). The challenge was considered positive if two or more signs and/or symptoms developed.

Bronchial Challenges

Single-blind bronchial challenges (BrC) were performed on 24 subjects in two groups. *Group 1-BrC* had ten extrinsic asthmatic subjects with symptoms during the BCE pollinating season and positive intradermal BCE skin tests; and *group 2-BrC*, 14 subjects with negative BCE skin tests (four with extrinsic asthma and positive prick tests to two or more common local

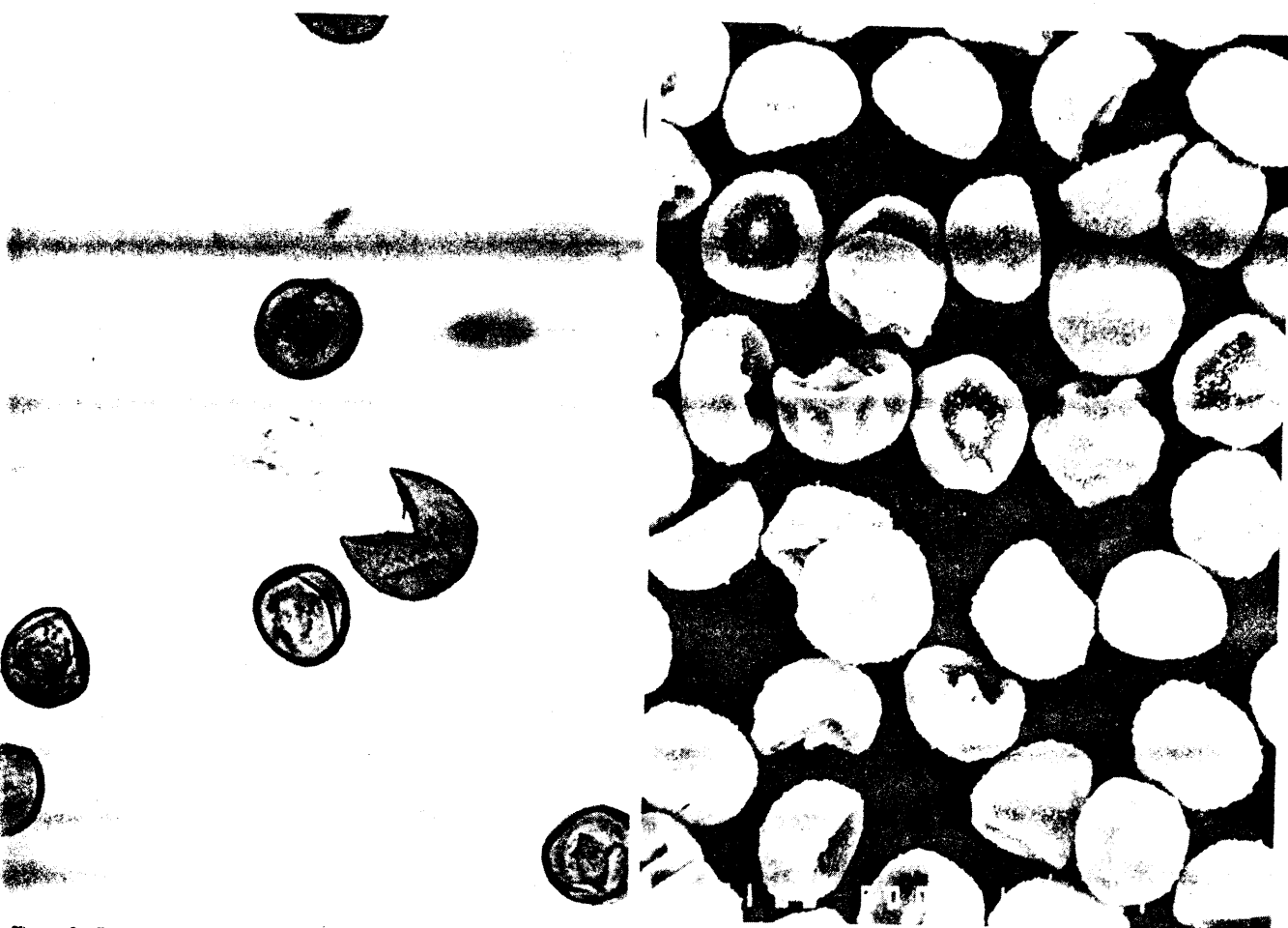


Figure 2. Bald cypress (*Taxodium distichum*) pollen grains. (left hand panel) Calberla's stain showing exine and expelled intine ($\times 400$); (right hand panel) scanning electron micrograph ($\times 600$), courtesy of A. Slater.

allergens, three with intrinsic asthma and negative skin test to common local aeroallergens, and seven normal subjects). Fifteen of 17 asthmatics who were tested had either a documented 15% reversibility of FEV_1 following an inhalation of a beta-2-agonist (ten subjects) or a positive (20% drop in FEV_1) methacholine challenge before or with five breaths of the final concentration of 25 mg/mL (five subjects) as described by Chai et al.¹⁴ Two extrinsic asthmatics in group 1-C did not return for bronchodilator or methacholine testing.

The antigen and methacholine bronchial challenge tests were performed using a Rosenthal-French nebulization dosimeter model D-2A and a DeVilbiss #646 nebulizer as described by Chai et al.¹⁴ A computerized Minis Eagle I spirometer was used for pulmonary function testing. Bronchial challenges were performed by on asymptomatic subjects with no respiratory tract infections within 4 weeks and a baseline FEV_1 which was 80% or greater of either the predicted FEV_1 or of the previous high FEV_1 . Subjects were not challenged if FEV_1 was less than 60% of predicted.

The dosimeter was set for 0.6 second nebulization

which delivered 0.016 mL of nebulized solution. The challenges were begun with five inhalations of diluent, ten minutes after which spirometry was repeated and the FEV_1 recorded as the control FEV_1 . The challenge proceeded if the control FEV_1 was greater than 90% of the baseline FEV_1 . The BCE inhalations were begun with five timed inhalations of the lowest concentration of BCE required to elicit a 6 to 8-mm wheal on intradermal skin testing. Spirometry was repeated ten minutes later. A 20% drop in the control FEV_1 sustained for 20 minutes was considered a positive immediate challenge. If the drop in FEV_1 was less than 20% at ten minutes, the next concentration was administered utilizing the following inhalation increments: 1:500,000, 1:100,000, 1:50,000, 1:10,000, 1:5,000, 1:1,000, and 1:500 wt/vol. A test was considered negative if the drop in FEV_1 was less than 20% at ten minutes after five inhalations of the 1:500 concentration. The subjects were instructed to report any delayed reactions and six of the ten patients in group 1-BrC were issued Mini-Wright peak flow meters to measure and record their peak flow at hourly intervals over the next 24 hours while awake. A 25% or greater drop of the peak flow

BALD CYPRESS TREE POLLEN — BUCHOLTZ ET AL

beginning at 1 to 24 hours after challenge was considered a positive delayed bronchial reaction.

Statistical Analysis

The Student's *t* test was used for statistical comparison between skin tests and challenges and between RAST and challenges. Regression analysis was applied to the skin test (mm) and RAST (percentage of TCBM).

Results

Nasal Challenge

Positive NCs were found in 12 of the 17 (71%) subjects (group 1-NC) with seasonal allergic rhinitis and positive intradermal skin test BCE. Negative NCs occurred in all of the 16 subjects (group 2-NC) with negative intradermal skin test with BCE (Table 1).

Bronchial Challenge

Positive bronchial challenge tests with BCE were found in two of the ten subjects (group 1-BrC) with extrinsic asthma and positive skin test with BCE. One patient had an immediate reaction. The other had a delayed reaction manifested by cough, dyspnea, and wheezing at five hours documented by a 31% drop in peak flow at seven hours post-challenge. Negative bronchial challenges were found in all 14 of the subjects (group 2-BrC) with negative skin tests with BCE (Table 1).

RAST

The RAST was done in 39 of 41 subjects. The sera of two subjects (one in group 1-BrC, one included in groups 2-NC and 2-BrC) were lost during storage and not done. The RAST results ranged from 0.76% to 24.1% of TCBM. The RAST results of the seven normal subjects sera ranged from 0.76% to 1.8% TCBM. The mean plus three standard deviations of the seven normals was 2.2% TCBM. RAST values greater than this were considered positive.

Thirteen of the 39 subjects sera tested had positive RAST. All 13 subjects with positive RAST had positive intradermal skin tests. A positive RAST was found in 13 of the 18 (72%) subjects with positive intradermal skin tests. Five of the 26 (19%) subjects with negative RAST had positive intradermal skin tests (Table 2). The bimodal power correlation between the skin tests

Table 2. Comparison of Skin Test and RAST in 39 Patients Using Crude Bald Cypress (*Taxodium Distichum*) Pollen Extract

	Skin Test Positive	Skin Test Negative
RAST positive	13	0
RAST negative	5	21

Table 3. Comparison of RAST and Challenge Tests Using Crude Bald Cypress (*Taxodium Distichum*) Pollen Extract

	RAST Positive	RAST Negative	P*
Nasal challenge positive	7/11 64%	5/20 25%	<.05
Nasal challenge negative	4/11 36%	15/20 75%	<.05
Bronchial challenge positive	1/8 13%	1/15 7%	.41
Bronchial challenge negative	7/8 87%	14/15 93%	0.44

* *P* ≤ .05 is significant.

(mm) and RAST (TCBM) had an *r* value of .697 with *f* = 19.5.

Agreement between RAST and skin tests (ST)

$$= \frac{[13 \text{ (RAST+, ST+)} + 21 \text{ (RAST-, ST-)}]}{39 \text{ subjects tested}} \times 100 = 87\%$$

Nasal challenges were positive in seven of 11 (64%) subjects with positive RAST and five of 20 (25%) subjects with a negative RAST.

Agreement between RAST and NC

$$= \frac{[7 \text{ (RAST+, NC+)} + 15 \text{ (RAST-, NC-)}]}{39 \text{ Subjects Tested}} \times 100 = 71\%$$

Bronchial challenges were positive in one of 8 (13%) subjects with positive RAST and one of 15 (7%) subjects with a negative RAST (Table 3).

Discussion

Skin test reactivity predicted nasal reactivity (71%, *P* < .001) and a negative skin test predicted lack of nasal reactivity (100%, *P* < .001). Skin test reactivity was a poor predictor of bronchial reactivity (20%, *P* < .33). None of the patients with a negative skin test had positive BrC.

Bruce et al found a good correlation between skin tests and BrC.¹⁵ They have shown, by linear regression analysis, that the provocation dose causing a 35% drop in specific airway conductance (PD₃₅ SGaw) of seasonal asthmatic patients sensitive to ragweed extract plotted against the degree of skin sensitivity had a correlation coefficient of 0.64 (*P* < .001). But in the study by C. Bruce et al. highly allergic asthmatics were challenged with a final dose of 0.88 mL of 1:1000 wt/vol ragweed pollen extract as compared with a final dose of 0.04 mL of 1:500 wt/vol BCE in the present study. Studies

Table 1. Comparison of Skin Tests and Challenge Tests Using Crude Bald Cypress (*Taxodium distichum*) Pollen Extract

	Skin Test Positive ≥8mm	Skin Test Negative <8mm	P*
Nasal challenge positive	12/17 71%	0/16 0%	<.001
Nasal challenge negative	5/17 29%	16/16 100%	<.001
Bronchial challenge positive	2/10 20%	0/14 0%	.33
Bronchial challenge negative	8/10 80%	14/14 100%	.29

* *P* ≤ .05 is significant.

by others have found that patients with tree pollen sensitivity are less reactive on BrC than patients with ragweed, dust, grass, and mold sensitivity.^{16,17} Spector and Farr found that asthmatic patients with positive intradermal skin tests (6-mm wheal) using 1:1000, 1:10,000, and 1:100,000 wt/vol tree pollen extracts had 27%, 8%, and 57% positive BrC (20% decrease FEV₁), respectively.¹⁶ This high correlation with skin test sensitivity was present in patients of considerably greater sensitivity than in this study and furthermore, the final challenge concentration used was much greater, ie, 40 inhalations of a nebulized 1:1,000 wt/vol tree pollen extract. Holman et al, using a tree pollen mixture, found 28.5% positive bronchial reactions (25% decrease peak expiratory flow rate) in patients with positive prick skin tests to the same tree pollen mixture.¹⁷ Thus, the 20% positive BrCs in skin test-positive subjects, although not statistically significant, is comparable to the results reported in these previous studies using tree pollen extracts.

The RASTs were positive in 72% of subjects with positive skin tests (Table 2). The skin tests were positive in all subjects with RAST positive sera. Nineteen percent of RAST-negative subjects had positive skin tests. There was an overall agreement of 87% between skin tests and RAST. This compares to 42% to 88% correlation between the skin test and RAST results in other studies.¹⁸⁻²⁰

A positive RAST predicted a positive NC in 64% of subjects challenged, while a negative RAST predicted a negative NC in 75% of subjects challenged. The agreement between RAST and NC is 71%. This is comparable to the 76% agreement found by Berg and Johanson.¹⁸ The RAST was not predictive of bronchial reactivity since only 1/8 (12.5%) of the subjects with positive RAST had a positive bronchial challenge.

There are a number of factors that may account for the lack of bronchial reactivity: (1) a weak antigen, (2) inadequate delivery of antigen, and (3) subject factors, ie, bronchial reactivity and atopic state. All subjects were retested intradermally within half an hour prior to BrC with the same extract that was used for the challenge. Any loss of antigenic potency should have been reflected in a loss of skin reactivity, and no loss of reactivity was observed (all extracts were used within 10 months of vialing). The delivery system was calibrated for output (0.016 mL with dosimeter set at 0.6 seconds) and was also used for methacholine challenges, most of which were positive. All subjects were cooperative and inhaled each dose slowly for at least five seconds and retained it for at least ten seconds prior to exhalation. All evidence would therefore indicate that the system delivered the extract properly to the lower respiratory tract. Possibly more positive bronchial challenges would have been found if a more concentrated final dilution, ie, 1:100 wt/vol instead of 1:500 wt/vol, were employed. Cockcroft et al have found that bronchial responsiveness to inhaled allergen correlates not

only with skin sensitivity, but also with the degree of nonallergic bronchial responsiveness.²¹ Two asthmatic subjects by history with positive BCE skin tests and negative BrCs with BCE were not tested for reversibility or methacholine reactivity. Two asthmatics in the same group had a positive methacholine challenge but reacted to one breath and two breaths of the final concentration (25 mg/mL). Thus, they had a relatively low state of bronchial reactivity. All other asthmatics had documented reversibility within 6 months prior to the BrC. Two (34%) of the remaining six asthmatics with proven reversibility and positive BCE skin tests had a positive BrC. All BrCs were performed at least 3 months out of season and therefore, subjects are expected to be less reactive. A minority of asthmatics, however, did display bronchial reactivity to BCE.

The bald cypress pollen is an aeroallergen and the skin test is a more sensitive indicator of clinical sensitivity than the RAST. The specific allergen(s) in bald cypress pollen is yet to be isolated or characterized and is currently being investigated.

Acknowledgments

We would like to thank Mrs. Lolita Binford for her technical assistance, and Mrs. Patricia A. Walsh and Ms. Peggy L. Hales for their secretarial assistance. We also thank Samuel C. Bukantz, MD for his editorial assistance in preparing this manuscript.

References

1. Lakela O, Long RW, Fleming G, et al: Plants of the Tampa Bay Area, Miami, 1979, University of South Florida, Banyan Books, p. 8.
2. Solomon WR, Mathew KP: Aerobiology and inhalant allergens, in Middleton E Jr, Reed CE, Ellis EF (eds): *Allergy Principles and Practice*, ed 2. St. Louis, Toronto, CV Mosby Co, 1983, vol 2, p 1162.
3. Baba M, Furuya K, Miyamoto T, et al: Japan, in Roth A (ed): *Allergy in the World*. Honolulu, The University Press of Hawaii, 1978, p 100.
4. Yasueda H, Yui Y, Shimizu T, et al: Isolation and partial characterization of the major allergen from Japanese cedar (*Cryptomeria japonica*) pollen. *J Allergy Clin Immunol* 1983;71:77.
5. Ordman D: Cypress pollinosis in South Africa. *S Afr Med J* 1945;19:142.
6. Tas J: Hay fever due to the pollen of *Cupressus sempervirens* Italian or Mediterranean cypress. *Acta Allergol* 1965;20:405.
7. Hobday J: The incidence of pollen sensitivities on skin test and bronchial challenge in asthmatic children in Perth, Western Australia. *Med J Aust* 1972;1:161.
8. Yoo TJ, Spitz E, McGerity J: Conifer pollen allergy: studies of immunogenicity and cross antigenicity of conifer pollens in rabbit and man. *Ann Allergy* 1975;34:87.
9. Serbousek D, Lockey RF, Binford L, et al: Aeroallergen Survey of the Tampa Bay Area. (Personal observation).
10. Newmark FM: Aeroallergens, in Lockey RF (ed): *Allergy and Clinical Immunology*. Garden City, Medical Examination Publishing Company, Inc, 1979, p 634.
11. Wodehouse RP: Pollen grains. New York, London, Harper Publishing Company, 1965, p 268.
12. Vanselow NA: Skin testing and other diagnostic procedures, in Sheldon JM, Lovell RG, Mathews KP (eds): *A Manual of Clinical Allergy*, ed 2. Philadelphia, London, WB Saunders Co, 1967, p 62.

BALD CYPRESS TREE POLLEN — BUCHOLTZ ET AL

13. Ceska MR, Eriksson R, Varga JM: Radioimmunosorbent assay of allergens. *J Allergy Clin Immunol* 1972;49:1.
14. Chai H, Farr RS, Froehlich LA, et al: Standardization of bronchial inhalation challenge procedures. *J Allergy Clin Immunol* 1975;56:323.
15. Bruce C, Rosenthal R, Lichtenstein L, et al: Diagnostic tests in ragweed or allergic asthma. *J Allergy Clin Immunol* 1974;53:230.
16. Spector S, Farr R: Bronchial inhalation procedures in asthmatics. *Med Clin N Am* 1974;58:71.
17. Holman J, Molk L, Micklich O: Bronchial challenge and skin test correlations. *Ann Allergy* 1972;30:250.
18. Berg TLC, Johansson SGO: Allergy diagnosis with the radioallergosorbent test. *J Allergy Clin Immunol* 1974;54:209.
19. Aas K, Johansson SGO: The radioallergosorbent test in the in vitro diagnosis of multiple reaginic allergy. *J Allergy Clin Immunol* 1971;48:134.
20. Pascual HC, Reddy Pm, Nagaya H, et al: Agreement between radioallergosorbent test and skin test. *Ann Allergy* 1977;39:325.
21. Cockcroft DW, Ruffin RE, Frith PA, et al: Determinants of allergen-induced asthma: dose of Allergen, circulating IgE antibody concentration, and bronchial responsiveness to inhaled histamine. *Am Rev Respir Dis* 1979;120:1053.

Requests for reprints should be addressed to:

Gerald A. Bucholtz, MD

University of South Florida College of Medicine

% VA Hospital

13000 N. 30th St (VAR 111D)

Tampa, FL 33612

"The mind like a sick body can be healed and changed by medicine."

Lucretius