

Occupational asthma caused by Brazil ginseng dust

Javier Subiza, MD, Jose Luis Subiza, MD,* Pedro Martin Escribano, MD, Miguel Hinojosa, MD, Rosario Garcia, MD, Miguel Jerez, and Eliseo Subiza, MD Madrid, Spain

The inhalation of different substances of plant origin can cause immediate and late onset asthma. The list of these agents responsible for such reactions is continuously increasing. We discuss a patient who developed symptoms of asthma after exposure to Pfaffia paniculata root powder used in the manufacturing of Brazil ginseng capsules. Airway hyperreactivity was confirmed by a positive bronchial challenge to methacholine. Sensitivity to this dust was confirmed by immediate skin test reactivity, a positive bronchial challenge (immediate response), and the presence of specific IgE detected by ELISA technique to an aqueous extract. The bronchial response was inhibited by sodium cromoglycate. Unexposed subjects did not exhibit reactivity to this ginseng extract with any of the tests referred to above. The same study performed with Korean ginseng (Panax ginseng) elicited negative results. This study is the first, to our knowledge, that links ginseng-root dust to occupational asthma. (J ALLERGY CLIN IMMUNOL 1991;88:731-6.)

Key words: Allergy, asthma, ginseng, occupational disease

Many inhaled organic agents can cause occupational asthma in atopic and nonatopic subjects. An important number of these agents are plant-derived material, and in many cases an immediate type I IgE-mediated hypersensitivity mechanism has been implicated. The sources of inducing Ags have been found in many different parts of the plant: grains, fruits, seeds, pollens, wood, plant exudate (some vegetable gums and latex), leaves, and roots.¹⁻⁶ Korean ginseng (*Panax ginseng*) is an herbal root that has been cultivated and used for centuries in the Orient, and numerous articles have been written concerning its medical stimulant and aphrodisiac properties. At the present time, large-scale ginseng cultivation is industrially processed in modern factories in Korea, producing millions of dollars worth of the root in the form of powder, liquid extract, creams, tablets, and capsules, which are sold worldwide.⁷ In contrast, the roots of *Pfaffia paniculata*, known as Brazil ginseng,

Abbreviations used

PBS:	Phosphate-buffered saline
BSA:	Bovine serum albumin
Ag:	Antigen
Ab:	Antibody
PC ₂₀ :	Provocative concentration a 20% drop in FEV ₁

have been used both as a tonic aphrodisiac and for antidiabetic purposes as a folk medicine. Collected in Brazil, it has a similar morphology and some of the properties of the Korean ginseng. Moreover, a new nortriperne (pfaffic acid), which exerts inhibitory effects on the growth of cultured tumor cells, has been isolated from these roots.^{8, 9} However, ginseng has also been demonstrated capable of causing adverse effects. Studies of acute hypertension after a short course of *Panax ginseng* treatment have been described,¹⁰ together with side effects of behavior stimulation, sleeplessness, diarrhea,¹¹ mastalgia,¹² and vaginal bleeding.¹³ Nevertheless, to the best of our knowledge, no allergic reactions and/or asthma have ever been described to be induced by Brazil or Korean ginseng. This article concerns a patient who developed an IgE-mediated sensitization to Brazil ginseng-root dust leading to rhinitis and asthma.

From the Centro de Alergia e Inmunología Clínica, General Pardiñas, Madrid, and *Servicio de Inmunología, Hospital Universitario San Carlos, Madrid, Spain.

Received for publication Dec. 31, 1990.

Revised June 3, 1991.

Accepted for publication June 3, 1991.

Reprint requests: Javier Subiza, MD, Centro de Alergia e Inmunología Clínica, General Pardiñas, c/ General Pardiñas 116, Madrid, 28006, Spain.

1/1/31506

CASE REPORT

A 37-year-old woman had worked alone in the bottling and packing of Brazil ginseng-root dust in a small laboratory for a year. Six months after she began working, she noticed runny nose, nasal and ocular itching, and sneezing. These symptoms occurred within 5 to 10 minutes of exposure to ginseng dust. She tried using a face mask to avoid contact with the ginseng dust and also started treatment with terfenadine, 60 mg, twice daily. After a few months of slight improvement, her condition became worse. She experienced additional symptoms of cough, wheezing, and shortness of breath. Consequently, she was referred to us for evaluation. On holidays the severity of her asthmatic symptoms decreased markedly until she finally remained completely asymptomatic. She is a nonsmoker and has no other history of respiratory or allergic diseases. There was no family history of atopy. The patient was not receiving medication when she was admitted to our center. The nasal mucosa was pale and edematous. Diffuse expiratory wheezes were noted over both lung fields. Results of sinus examination and chest x-rays were normal. White blood cell count was 5900 cells per cubic millimeter, of which 7% were eosinophils. Total IgE serum was 200 IU/ml. Initial spirometry revealed an FEV₁ of 2430 ml (85% predicted) that increased to 3180 ml (111% predicted) after inhalation of salbutamol (0.2 mg).

MATERIAL AND METHODS

Preparation of ginseng root-dust extract

Four grams of the patient's Brazil ginseng-root dust, *Pfaffia paniculata* (Emperor, Brazil) were defatted with acetone, dried, and extracted in PBS (40 ml of 0.01 mol/L of phosphate and 0.15 mol/L of NaCl, pH 7.4), and the mixture (1:10 wt/vol) was stirred for 12 hours at 4° C. The suspension was filtered through a filter paper (Whatman Ltd., Maidstone, England), dialyzed against PBS, and sterilized by 0.22 µm filtration (Millipore, Molsheim, France), having a final concentration of 345 µg/ml in protein, as determined by Bio-Rad protein assay (Bio-Rad Laboratories, Richmond, Calif.). The solution was aliquoted and stored at -20° C until use. The same procedure was used to prepare an extract with Korean ginseng-root powder, *Panax ginseng* (Ginseng Corp., Seoul, Korea), and with *Dermatophagoides pteronyssinus* (α-base, Dome-Hollister-Stier, Bridgend, UK).

Skin tests

All skin tests were performed by the prick technique, beginning with a dilution of 1:1,000,000 wt/vol of the ginseng extracts and were progressively increased by tenfold until a positive reaction was obtained.

A battery of commercially available common inhalants and food allergens (Abello Laboratory, Madrid, Spain) were also prick tested.

Five patients with hay fever and five normal individuals were tested with the ginseng extract as a control. Histamine phosphate (1 mg/ml) was used as a positive control, and 50% glycerol was used as a negative control. All skin test sites were read after 15 minutes and 4 to 8 hours later. A positive reaction was defined as a wheal of at least 3 mm

by 3 mm in the presence of a negative reaction to the 50% glycerol and a positive test with histamine phosphate.

Precipitin test

The patient's serum was tested for precipitating Abs to undiluted Brazil ginseng extract (1:10 wt/vol) by Ouchterlony diffusion gels.¹⁴

ELISA

This test was performed as previously described.¹⁵ Briefly, the wells of a polystyrene 96-well microplate (Nunc, Roskilde, Denmark) were coated with both ginseng extracts at a protein concentration of 10 µg/ml in PBS. After an overnight incubation at 4° C, the plates were washed (PBS and 0.1% Tween 20) and neutralized with 1% BSA (Sigma Chemical Co., St. Louis, Mo.) in PBS (PBS-BSA) for 1 hour at room temperature. After an additional wash, 100 µl of undiluted serum was added per well, and the plates were incubated 4 hours at room temperature. Then, the wells were washed and filled with 100 µl of 1:4000 dilution (PBS-BSA) of affinity-isolated goat F(ab')₂ Abs to human ε chain fragments and conjugated with peroxidase (Tago, Inc., Burlingame, Calif.). After an overnight incubation at 4° C and a final washing step, 100 µl of a substrate solution (0.05% of H₂O₂, 0.02 mol/L of o-phenylenediamine, and 0.1 mol/L of citrate buffer, pH 5.5) was added. The enzymatic reaction was allowed to develop for 10 minutes and then stopped with 100 µl of 2 N HCl. The absorbance values were measured at 492 nm (SLT 210; Kontron, Grodig, Austria), and the results were expressed in optical density. ELISA was also used to carry out inhibition assays as previously described in detail.¹⁵ Both ginseng extracts and unrelated Ag (*D. pteronyssinus* extract), adjusted at 0.2 to 200 µg/ml in protein, were used as inhibitors.

Bronchial provocation tests

Bronchial challenges with methacholine and Brazil ginseng extract were performed according to the method of Cockcroft et al.¹⁶ and Juniper et al.¹⁷ with some modifications previously described.^{5,6} These tests were performed during an asymptomatic period after 7 days without exposure to ginseng dust. The following modifications were used: The aerosol was generated in all instances by the same DeVilbiss 646 nebulizer (DeVilbiss Co., Somerset, Pa.) with an output of 0.28 ml/min, delivered into a mask held loosely over the subject's mouth and with a noseclip over the nose. The patient inhaled the solution at tidal breathing for 2 minutes, starting with PBS solution as a control, followed by an increasing concentration of methacholine, 0.04, 0.09, 0.19, 0.39, 0.78, 1.56, 3.12, 6.25, 12.5, and 25 mg/ml, at intervals of 6 minutes. Measurements of FEV₁ and FVC were made with a precalibrated Vitalograph-PFT spirometer (Vitalograph Ltd., Buckingham, England) before the test and at 30 seconds and every 4 minutes after each inhalation. The test was terminated when the FEV₁ had fallen by 20% or more from the post-PBS level. The result was expressed as the provocative concentration of methacholine required to induce PC₂₀ that was interpolated from the dose-response curve.

TABLE I. IgE reactivity with Brazil and Korean ginseng extracts in patient's serum detected by ELISA

ELISA wells coated with	Patient's serum		Control sera	
	Unheated	Heated*	Healthy†	Hay fever‡
None	0.02	ND	ND	ND
Brazil ginseng	1.21	0.15	0.12 ± 0.05	0.12 ± 0.10
Korean ginseng	0.14	ND	0.16 ± 0.09	0.18 ± 0.07

ND, Not done.

Results are expressed in optical densities at 492 nm (mean ± SD, except patient's data that correspond to the mean of duplicates).

*Four hours at 56° C.

†From 16 nonatopic, control subjects.

‡Sera from 11 patients with hay fever with serum IgE level between 500 to 1000 IU/ml.

Bronchoprovocation testing with Brazil ginseng extract was performed as for the methacholine inhalation tests but in a double-blind protocol. A control test day, with exposure to a PBS inhaled for 2 minutes, was performed to ensure that the patient's FEV₁ was stable during at least 8 hours and that any possible physiologic changes observed on challenge days were not attributable to normal circadian changes in pulmonary function or changes in effort.¹⁸ Different dilutions of Brazil ginseng extract were inhaled by the patient. PBS was used as placebo. The highest Brazil ginseng-extract concentration prepared for patient's inhalation tests was 1:100 wt/vol; therefore, there would not be a significant difference in color, odor, or taste between these dilutions and the PBS. A member of the staff randomized all challenges so that the investigators and patient were unaware of the content of any challenge. FEV₁ was measured before and at intervals of 5 minutes after each inhalation for the first 30 minutes, at 1 hour, then hourly for the next 8 hours, and again at 24 hours. Inhalation was stopped when there was a fall in FEV₁ of 20% or more from PBS inhalation control. The following inhalation tests were conducted on different days: Brazil ginseng extract in stepwise concentration of 1:1,000,000, 1:100,000, 1:10,000, and 1:1000 wt/vol. Two unexposed patients with asthma were also challenged but in a single-blind manner with a 1:10 wt/vol dilution after informed consent was obtained. Sodium cromoglycate (40 mg) was administered to the patient by inhalation with a Spinhaler (Fisons Corp., Bedford, Mass.) 30 minutes before a challenge test with a 1:1000 wt/vol Brazil ginseng extract.

RESULTS

Skin tests

The patient exhibited a 3 by 3 mm wheal with erythema 15 minutes after prick testing with the 1:10,000 wt/vol dilution of Brazil ginseng extract. The 1:1000 dilution elicited increasing local reaction with 7 by 4 mm wheal with pseudopods. A more concentrated dilution was not tested. No skin late reaction was observed with any of the dilutions. In contrast, the five atopic and five normal subjects did not react to the 1:10 wt/vol dilution. The patient

did not have a positive reaction to either common inhalants, food allergens, or Korean ginseng extract.

Specific IgE measurement

To confirm the presence in this patient of IgE Abs to Ags contained in Brazil ginseng extract, we tested the patient's serum for specific IgE by ELISA. As presented in Table I, there was IgE activity to Brazil ginseng, but not to Korean ginseng extract. IgE activity decreased to background levels when the patient's serum was first heated, as expected by the nature of the Abs detected. IgE activity to this extract was not detectable in serum samples from 16 healthy individuals or from 11 patients with hay fever (Table I) containing high levels of serum IgE. To support the lack of serum IgE reactivity with Korean ginseng in this patient, inhibition assays were performed with both ginsengs as inhibitors (see Fig. 2). As can be observed, IgE reactivity with Brazil ginseng could not be inhibited with the highest concentration of Korean ginseng extract tested (200 µg/ml), or with *D. pteronyssinus* extract used as an unrelated Ag, whereas this reactivity was readily absorbed with Brazil ginseng as inhibitor (Fig. 2).

Precipitin tests

Abs to Brazil ginseng extract were not observed in our patient serum, as detected by double-diffusion gels.

Bronchial provocation tests

Methacholine challenge demonstrated slight bronchial hyperresponsiveness (methacholine PC₂₀, 1.8 mg/ml). On the control day of exposure to PBS, there was no significant change in spirometry throughout the day. In contrast, the patient had an immediate asthmatic response after challenge with the 1:1000 wt/vol dilution of the Brazil ginseng extract. As de-

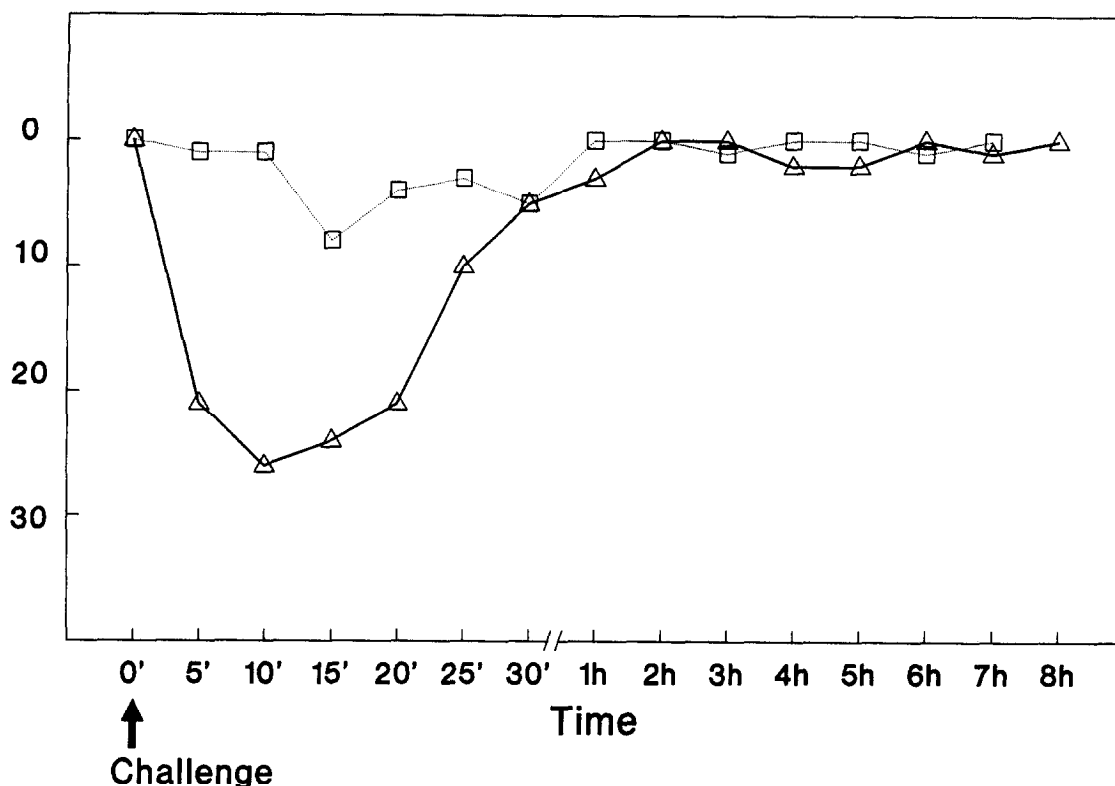
Percent Decrease of FEV₁

FIG. 1. Bronchial provocation test (Δ) with 1:1000 wt/vol Brazil ginseng extract. The same test (□) performed 30 minutes after cromolyn sodium inhalation.

picted in Fig. 1, at 10 minutes, there was a 26% fall in FEV₁ that returned to the baseline approximately 2 hours later. No late reaction was observed. The immediate reaction was inhibited by previous inhalation of sodium cromoglycate (Fig. 1). The two unexposed subjects with asthma demonstrated a methacholine PC₂₀ of 1.2 and 1.9 mg/ml. In contrast, subjects did not react to the 1:10 wt/vol Brazil ginseng extract.

DISCUSSION

Our patient experienced asthma within a few months after starting to package Brazil ginseng-root dust. Furthermore, the onset of nasal and respiratory symptoms within minutes of exposure to ginseng dust at work and the noticeable improvement while she was away from work during vacation indicate the occupational nature of the asthma. It may be argued, however, that in view of her positive methacholine challenge, the subject had preexisting asthma and that the dusty atmosphere at work simply aggravated her underlying condition. Bronchial asthma is known to be associated with hyperreactivity of airways to diverse nonspecific stimuli and to drugs, such as methacholine.¹⁶⁻¹⁹ However, the negative bronchial response to PBS in the patient and the negative bronchial re-

sponse to the highest concentration (1:10 wt/vol) of Brazil ginseng extract in two unexposed subjects with asthma with similar nonspecific bronchial hypersensitivity denote in the patient a true specific immediate bronchospastic reaction to the 1:1000 wt/vol Brazil ginseng extract. Moreover, this bronchospastic reaction was greatly reduced by previous inhalation of cromolyn, which might suggest an IgE-mediated mechanism to be operational in the underlying cause of the ginseng-induced asthma. These conclusions are supported by the presence of an immediate response in the prick tests and by the presence of specific IgE to the Brazil ginseng extract. The negative results obtained in a group of unexposed normal and atopic subjects support the specificity of these findings.

Our results also demonstrate that no IgE reactivity with Korean ginseng was detectable in patient's serum. Moreover, an extract of this latter was unable to inhibit IgE reactivity with Brazil ginseng. This lack of cross-reactivity is not surprising because of the lack of close taxonomic relation between both types of ginseng. Brazil ginseng (*Pfaffia paniculata*) belongs to the Amaranthaceae family; in contrast, the Korean ginseng (*Panax ginseng*) belongs to the Araliaceae family, which is included in a very distant phylogenetic

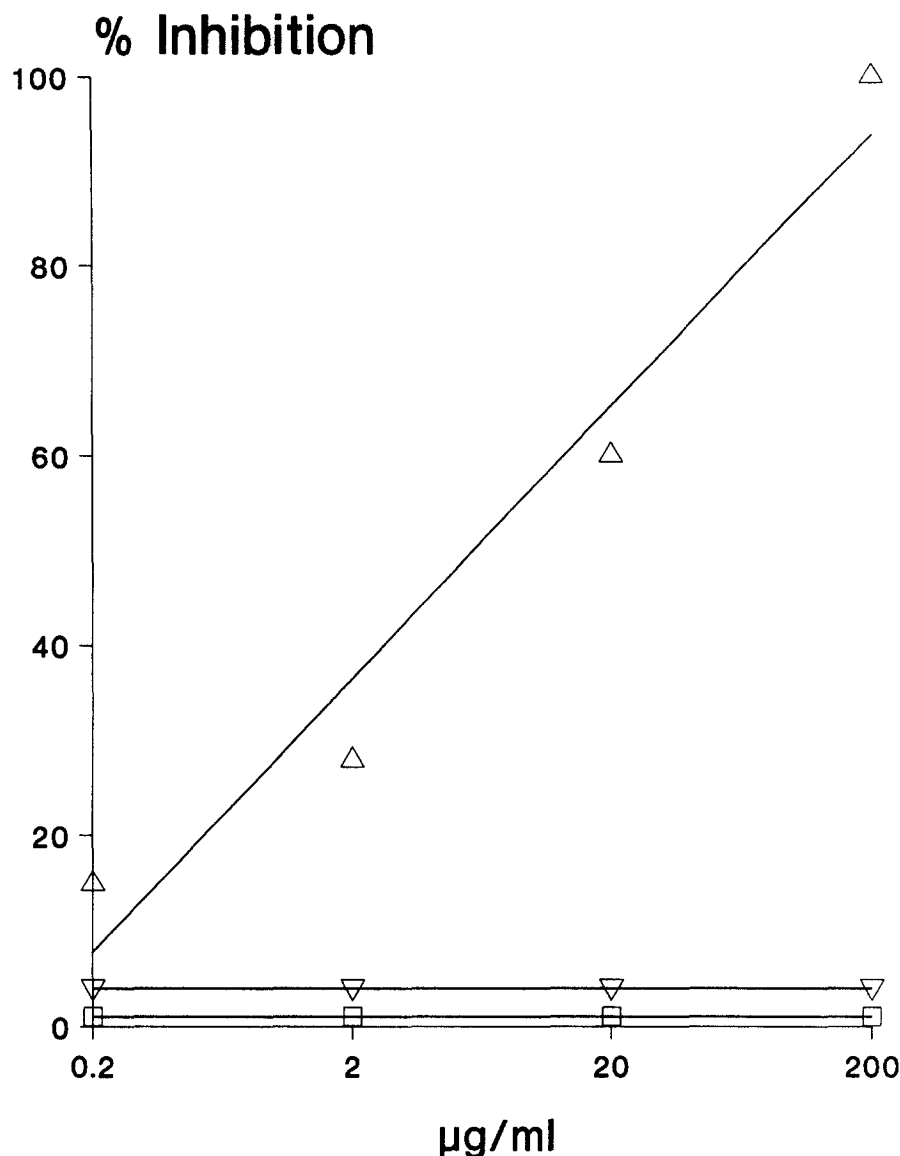


FIG. 2. Brazil ginseng ELISA-inhibition assay. Percent of inhibition of patient serum by increasing amounts of Brazil ginseng (Δ), Korean ginseng (∇), and one unrelated Ag (*D. pteronyssinus*) ($\square$). Note that IgE reactivity with Brazil ginseng cannot be inhibited with these two last Ags.

group.²⁰⁻²¹ It should be noted that the possibility of a contaminant accounting for the antigenicity of Brazil ginseng is very unlikely, since we searched very carefully for the presence of spores and pollen grains by microscopy to rule out this possibility. In contrast, symptoms appeared in our patient with Brazil ginseng from different batches.

The persistence of asthma while a protective mask was used suggests small diameter-sized dust as the carrier of the allergen(s). In contrast, the Ag(s) must be of macromolecule size because the Ag does not cross a dialysis membrane that should retain compounds with molecular weight >6000 to 8000 daltons. In this sense, treatment of Ag-coated ELISA wells

with pronase (1 mg/ml, Boehringer-Mannheim, Mannheim, Germany) but not with sodium metaperiodate (5 mmol/L) completely inhibited the patient's IgE reactivity (not presented), indicating that the antigenic determinants were proteins but not carbohydrate in nature.

In conclusion, the results of the investigation performed demonstrate that Brazil ginseng dust is a health hazard as an Ag able to induce asthma. An IgE-mediated immunologic mechanism appeared to be operational in the underlying pathogenesis.

We thank Jayne Kapastasy for preparation of the manuscript.

REFERENCES

- Berstein DI, Bernstein IL. Occupational asthma. In: Middleton E, Reed CE, Ellis EF, et al. eds. Allergy: principles and practice, vol 2. 3rd ed. St. Louis: CV Mosby, 1988:1197-1218.
- Butcher BT, Salvaggio JE. Occupational asthma [CME article]. J ALLERGY CLIN IMMUNOL 1986;78:547-56.
- Chan-Yeung M, Lam S. Occupational asthma. Am Rev Respir Dis 1986;133:686-703.
- Tarlo SM, Wong L, Roos J, Booth N. Occupational asthma caused by latex in a surgical glove manufacturing plant. J ALLERGY CLIN IMMUNOL 1990;85:626-31.
- Hinojosa M, Moneo I, Dominguez J, Delgado E, Losada E, Alcover R. Asthma caused by African maple (*Triplochiton scleroxylon*) wood dust. J ALLERGY CLIN IMMUNOL 1984;74:782-6.
- Hinojosa M, Moneo I, Cuevas M, Díaz-Mateo P, Subiza J, Losada E. Occupational asthma caused by *Voacanga africana* seed dust. J ALLERGY CLIN IMMUNOL 1987;79:574-8.
- Chong SKF, Oberholzer VG. Ginseng—is there a use in clinical medicine? Postgrad Med J 1988;64:841-6.
- Takemoto T, Nishimoto N, Nakai S, et al. Pfaffic acid, a novel nortriterpene from *Pfaffia paniculata* kuntze. Tetrahedron Lett 1983;24:1057-60.
- Nishimoto N, Nakai S, Takagi N, et al. Pfaffosides and nortriterpenoid saponins from *Pfaffia paniculata*. Phytochemistry 1984;23:139-42.
- Siegel RK. Ginseng abuse syndrome: problems with the panacea. JAMA 1979;241:1614-5.
- Brunner J. Nasty medicine [Letter]. N Scientist 1983;97:44.
- Palmer BV, Montgomery ACV, Monteiro JCMP. Ginseng and mastalgia [Letter]. Br Med J 1978;1:1284.
- Hopkins MP, Androff L, Benninghoff AS. Ginseng face cream and unexplained vaginal bleeding. Am J Obstet Gynecol 1988;159:1121-2.
- Ouchterlony O. Antigen-antibody reactions in gels: types of reactions in coordinated systems of diffusion. Acta Pathol Microbiol Immunol Scand 1953;32:231-4.
- Subiza J, Subiza JL, Hinojosa M, et al. Anaphylactic reaction after the ingestion of chamomile tea: a study of cross-reactivity with other composite pollens. J ALLERGY CLIN IMMUNOL 1989;84:353-8.
- Cockcroft DW, Killian DN, Mellon JJA, Hargreave FE. Bronchial reactivity to inhaled histamine: a method and clinical survey. Clin Allergy 1977;7:235-43.
- Juniper EF, Frith PA, Dinnet C, Cockcroft DW, Hargreave FE. Reproducibility and comparison of response to inhaled histamine and methacholine. Thorax 1978;33:705-10.
- Cartier A, Bernstein IL, Burge PS, et al. Guidelines for bronchoprovocation on the investigation of occupational asthma: report of the Subcommittee on Bronchoprovocation for Occupational Asthma. J ALLERGY CLIN IMMUNOL 1989;84:823-9.
- Reed CE. Abnormal autonomic mechanism in asthma. J ALLERGY CLIN IMMUNOL 1974;53:34-41.
- Heywood VH. Las plantas con flores. Barcelona: Reverté, 1985:213-15.
- Lewis WH, Vinay P, Zenger VE. Airborne and allergenic pollens of North America, Baltimore and London: The Johns Hopkins University Press, 1983:1-6.

AVAILABLE NOW! The FIVE-YEAR (1981-1985) CUMULATIVE INDEX TO THE JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY can be purchased from the Publisher for \$42.00. This comprehensive, 156-page reference guide is a current presentation of all topics included in the JOURNAL from January 1981 to December 1985 (volumes 67-76)—the past 10 volumes. It incorporates complete references to over 672 original articles, abstracts, case reports, letters, editorials, and CME articles. It features 1,525 Subject Headings, under which there are 5,316 references. Each subject entry lists the complete article title, author(s), volume, page, and year of publication. It also includes 4,780 Author Entries, listing contributors, along with their respective titles, author-to-author referral, volume, page, and publication date.

To purchase, call or write: Mosby-Year Book, Inc., 11830 Westline Industrial Dr., St. Louis, MO 63146-3318, or telephone FREE 1-800-325-4177, Subscription Services, ext. 4351, or 314-453-4351. Please reference book code number 2605-6 when placing your order. PREPAYMENT REQUIRED. Make checks payable to Mosby-Year Book, Inc. (All payments must be in US funds drawn on a US bank.) Price: \$42.00 in the US, \$44.75 international (price includes mailing charges).