

Occupational asthma caused by *Voacanga africana* seed dust

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We report a case of a nonatopic patient in whom exposure to Voacanga africana (VA) dust precipitated asthma. The patient was indirectly exposed to this dust by her husband, a chemist working in a pharmaceutical plant in which VA is used for the production of vinburnine, an alkaloid derivative. Vinburnine (Cervoxan) is widely used in conditions associated with cerebral circulatory insufficiency. Studies revealed the presence of immediate skin test reactivity to VA dust, and specific anti-VA antibodies were detected in the patient's serum by the reverse enzyme immunoassay technique. Bronchial challenge with a VA extract also resulted in an immediate asthmatic response without late reaction. These findings suggest a type I IgE-mediated immunologic mechanism as being responsible for the patient's respiratory symptoms. Unexposed persons did not exhibit reactivity to this seed with any of the tests referred to above. To the best of our knowledge, this is the first reported case of occupational asthma caused by VA seeds. (J ALLERGY CLIN IMMUNOL 1987;79:574-8.)

A number of different types of dusts reported to cause occupational asthma are of vegetable origin. These include such substances as grain dust, green coffee bean dust, castor bean dust, soybean dust, wheat and rye flour dust, powdered gums of acacia, karaya, and tragacanth (used in printing), garlic dust, maiko (a dust from tuberous root of devil's tongue used in the production of the Japanese food konnyaku), and psyllium seed dust (used in the production of bulk laxatives).¹⁻⁴

This article concerns a patient in whom asthmatic symptoms were induced indirectly by her husband, a chemist occupationally exposed to VA. The seed is of considerable industrial importance used for obtaining vinburnine (Cervoxan; Beecham Laboratories, Bristol, Tenn.), an alkaloid widely used in conditions associated with cerebral circulatory insufficiency.

CASE REPORT

The patient, a 39-year-old housewife, was a nonsmoker and married to a chemist who had worked for the last

Abbreviations used

VA:	<i>Voacanga africana</i>
REIA:	Reverse enzyme immunoassay
PBS-TW:	Phosphate-buffered saline-0.1% Tween 20

5 years in a pharmaceutical plant in which VA seeds are used for the production of vinburnine.

Three years previously, she began to notice a runny nose, nasal and ocular itching, sneezing, cough, wheezes, and shortness of breath. There was a significant improvement in her symptoms at weekends and when her husband was away from the plant. On holidays the severity of her asthmatic symptoms decreased markedly until she finally remained completely asymptomatic. She had no other history of respiratory or allergic diseases, and there was no family history of atopy.

A few weeks after her symptoms had begun, she consulted her general practitioner who diagnosed intrinsic asthma and prescribed therapy with theophylline and corticosteroids. This therapy provided good relief of her symptoms. Recently, she consulted an allergist who suspected occupational asthma and referred her to our department for evaluation.

The patient was not receiving medication when she was admitted to the hospital. The only abnormal physical finding was scattered wheezes on chest auscultation. The white blood cell count was 8600 cells per cubic millimeter, of which 8% were eosinophils. Total IgE serum was 150

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TABLE I. Specific anti-VA IgE antibodies by REIA

	REIA value (OD ₄₉₂)*
Patient serum	1.665
Patient heated serum	0.050
20 Control sera	0.080 ± 0.020
Blank	0.050 ± 0.010

*Optical density at 492 nm.

IU/ml. Chest x-ray films were normal. Spirometry revealed a FEV₁ of 87% predicted, which increased to 130% after inhalation of salbutamol.

MATERIAL AND METHODS

Seeds of VA were ground until a fine dust was obtained. Four grams of this dust was added to 20 ml of PBS, pH 7.4, and the mixture was stirred at room temperature for 1 hour. The suspension was filtered through a filter paper, and the filtrate was dialyzed against PBS at laboratory temperature overnight and finally passed through a Millipore (Millipore Corp., Bedford, Mass.) filter for sterilization, having a final concentration of 1:20 w/v. Tenfold dilutions were made for skin and inhalation testing.

Skin tests

All skin tests were performed by the prick technique beginning with a dilution of 1:200,000 w/v of the VA crude extract and were progressively increased tenfold until a positive reaction was obtained.

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

The patient's husband, 10 patients with hay fever, and 10 normal individuals were tested with a 1:20 VA extract dilution as a control. All skin tests were examined after 20 minutes and 4 to 8 hours later.

Precipitin tests

The patient's serum was tested for precipitating antibodies to several VA extract dilutions by an agar gel diffusion method.⁵

Preparation of VA extract for in vitro assays

Ten grams of VA dust was defatted with acetone, dried, and extracted in 20 ml of 0.15 mol/L of borate buffer, pH 8, at room temperature for 2 hours. The supernatant, obtained after centrifugation (1500 × g for 30 minutes), was passed through a Millipore filter. The total concentration of proteins of this final solution determined by the Bio-Rad protein assay (Bio-Rad Laboratories, Richmond, Calif.) was 0.78 mg/ml. The solution was aliquoted and stored at -20° C until it was required.

REIA for IgE against VA extract

Specific IgE in the patient's serum was measured according to REIA, as described in the literature.⁶⁻¹¹

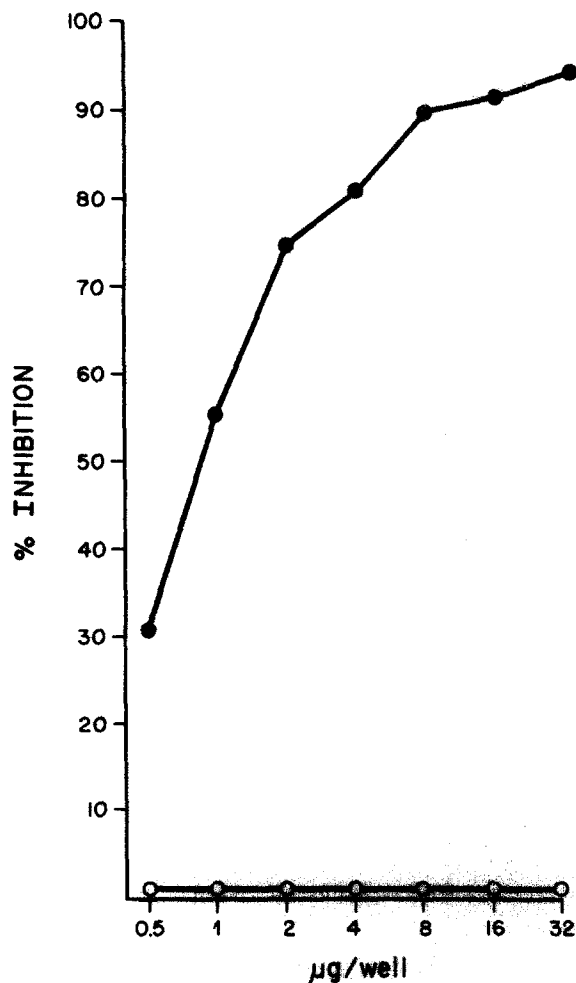


FIG. 1. VA REIA-inhibition assay: percent of inhibition of patient serum by increasing amounts of VA (●) and three unrelated antigens (○) (*O. europea*, *L. perenne*, and mustard seed).

Labeling procedure. One milligram of periodate oxidized peroxidase was mixed with 0.05 ml of the extract together with 0.05 ml of carbonate-bicarbonate buffer (1 mol/L, pH 9.6) and kept at 4° C for 8 hours. One milligram of sodium borohydride was then added, and the mixture was dialyzed against PBS for 24 hours at 4° C. Finally, 2% bovine serum albumin in PBS, containing 50% glycerol, was added until a final volume of 10 ml was obtained. This stock solution was aliquoted and stored at 4° C.

REIA assay. The patient's serum and the sera of 10 atopic and 10 nonatopic individuals, used as control subjects, were diluted in half in PBS-TW (0.1 ml per well) and incubated at 4° C for 18 hours in M 24 AR microplates (Dynatech Co., Alexandria, Va.) coated with monospecific antihuman IgE (Tago Inc., Burlingame, Calif.) as previously described.⁷

After several washes, 200 µl of a dilution of the labeled extract made in PBS-TW 25% fetal calf serum, containing 1 µg of peroxidase, was added to each well and left shaking for 1 hour at laboratory temperature. After 10 alternative washings with PBS-TW and tap water, the substrate (0.1

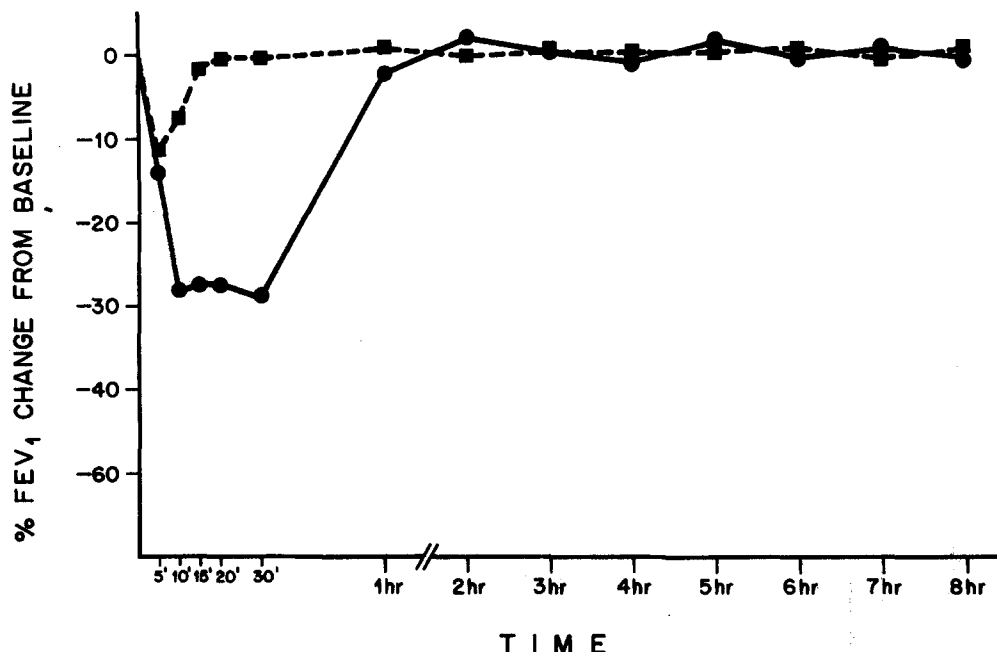


FIG. 2. Bronchial provocation test (●) with 1:200 w/v VA extract. The same test (■) performed 30 minutes after cromolyn sodium inhalation.

ml per well) was added and left for 30 minutes. The reaction was arrested with 2 N H₂SO₄, and the plates were read on Titertek Multiskan (Flow Laboratories, Irvine, Scotland) at an optical density at 492 nm. The substrate was composed of 1,2-phenylenediamine (2 mg/ml) in citrate phosphate buffer (0.1 mol/L, pH 5, 0.03% H₂O₂).^{7,9,10}

Specificity controls. The specificity of the VA REIA assay was tested by REIA-inhibition assay in the following manner: The patient's serum was incubated as described. After washing, the same amount of the conjugate was mixed with increasing amounts of the nonlabeled VA extract or three unrelated allergens (an *Olea europaea* pollen aqueous extract, 1:200 w/v, Abelló Laboratory, and either a *Lolium perenne* and mustard seed aqueous extracts, 0.043 mg/ml and 0.9 mg/ml, respectively, prepared by the authors) and added to the wells for 1 hour at room temperature while the mixture was left shaking. The assay was then continued as described above.

The specificity for IgE was assessed by heating the patient's serum at 56° C for 2 hours, and the assay was then continued as described above.

Bronchial provocation test

The patient had inhalation tests by a previously described method^{12,13} during an asymptomatic period when her husband had been on holiday for 3 weeks. 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 0.30 ml/min, delivered into a mask held loosely over the subject's mouth and with a noseclip over the nose, and inhaled by tidal breathing

for 2 minutes. FEV₁ was measured before and at intervals of 5 minutes after each inhalation for the first 30 minutes, at 1 hour, and later on, hourly, for the next 8 hours. Inhalations were stopped when there was a fall in FEV₁ of >20%, and challenges with the seed extracts were preceded by a control inhalation with PBS, pH 7.3, which caused no reaction.

The following inhalation tests were conducted on different days: VA extract in stepwise concentration of 1:200,000, 1:20,000, 1:2000, and 1:200 w/v. Two unexposed patients with asthma and two normal subjects were also challenged with a 1:20 w/v dilution after obtained informed consent.

The effect of disodium cromoglycate on the inhalation reaction was also studied. Cromolyn sodium (40 mg) was administered to the patient by inhalation with a Spinhaler (Fisons Corp., Bedford, Mass.) 30 minutes before a challenge test with a 1:20 w/v VA dilution.

RESULTS

Skin tests

A positive immediate reaction occurred in the patient after skin prick testing with the 1:200,000 w/v dilution (3 mm wheal and 6 mm flare). The 1:20,000 and 1:2000 w/v dilutions elicited increasing local reactions with a 11 by 7 wheal and erythema response with pseudopods resulting from the 1:200 w/v dilution. More concentrated dilutions were not tested. In contrast, the patient's husband, 10 atopic individuals, and 10 normal control individuals did not react to the 1:20 w/v dilution. Neither the patient nor the 10 nor-

mal control subjects had positive skin reactions to the commercial common allergens battery. The 10 atopic control subjects had positive reactions with several grass pollens and/or house dust mites and cat dander.

Precipitin tests

Precipitins to different dilutions of the VA crude extract were not found in the patient serum.

REIA and REIA-inhibition assays

Table 1 presents the optical density at 492 nm, obtained with the patient's serum as compared with 20 control sera or the patient's heated serum. A significant amount of specific IgE antibody was exhibited by the patient's serum. Fig. 1 illustrates that the REIA values can be inhibited by unlabeled VA, whereas there was no inhibition with the three unrelated allergens.

Bronchial provocation tests

The patient had an immediate asthmatic response after challenge with the 1:200 w/v dilution of the VA extract.

As depicted in Fig. 2, at 10 minutes there was a 28% fall in FEV₁ that returned to baseline approximately 90 minutes later. The patient noted chest tightness and overt wheezing. No late reaction was observed. Preinhalation of cromolyn sodium partially, but significantly, modified the reaction (Fig. 2). Unexposed patients with asthma and normal subjects did not react to the 1:20 w/v VA extract.

DISCUSSION

The clinical finding and results of the studies performed in our patient support an IgE-mediated immunologic mechanism to be operational as the underlying cause of the VA-induced asthma. Skin tests and bronchial challenges evinced an immediate response but no late reaction. This immediate reaction could be markedly inhibited by pretreatment with disodium cromoglycate. Furthermore, the presence of specific IgE antibodies in the patient's serum was demonstrated by the REIA study. On the contrary, the control group composed of unexposed atopic and normal individuals did not respond to skin, REIA, nor inhalation tests. These findings demonstrate the specificity of our patient response. Moreover, the REIA-inhibition study confirmed the specificity of the assay for VA antigens.

It is noteworthy that our patient, a nonatopic, middle-aged woman could become sensitized by the minimal amounts of the VA dust inhaled from her husband's clothing. This fact would appear to suggest

that antigen present in this seed possesses great sensitizing capacity. It is surprising that at the time of writing, we have found no other cases described of occupational allergy caused by VA seed dust. Unfortunately, the factory management denied us permission to carry out a survey on the more intensively exposed factory workers.

Exposure to VA seed dust should be considered a health hazard for which prevalent studies with the use of peak flow recordings, skin tests, and in vitro tests for specific antibodies are necessary.

The antigen(s) responsible for the occupational asthma described in this study are not characterized at present. However, the antigen does not cross a dialysis membrane that should retain compounds with molecular weight larger than 6000 to 8000 daltons and therefore must be a macromolecule. The antigen has been satisfactorily labeled with peroxidase, which demonstrates the presence of free amino groups and could lead to the belief that it probably is a protein.

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Role of the quaternary ammonium ion determinants in allergy to muscle relaxants

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Anaphylaxis to muscle relaxants appears to be a very useful model to study the IgE-dependent mechanisms of mediator release in humans. The serum IgE binding sites of the drugs appeared to be the ammonium ion determinants. In patients allergic to suxamethonium, one of the most frequently used muscle relaxants for general anesthesia, significant histamine release could be obtained in each case with simple diammonium salts. The length of the chain linking the ammonium groups appears to play an important role. In fact, when the length was ≤ 4 Å, no significant histamine release could be obtained, whereas the optimal length for histamine release appeared to be ≥ 6 Å. Furthermore, muscle relaxants with a rigid backbone between the ammonium determinants (such as pancuronium) are less active than flexible molecules (such as suxamethonium) in initiating mediator release. This study suggests that small divalent molecules can induce anaphylactic shock in sensitized patients and that the length and the flexibility of the chain bearing the haptenic determinants appear to be important factors in the elicitation of mediator release. (J ALLERGY CLIN IMMUNOL 1987;79:578-84.)

In the course of the 20-year period from 1964 to 1984, about 1000 anaphylactic reactions during general anesthesia were recorded in the world, and it was found that muscle relaxants were responsible for 51% of these adverse reactions.¹

Muscle relaxants were recently demonstrated to be able to act as true allergens with specific release of mediators in allergic patients in vivo and in vitro.²⁻⁴ Furthermore, the possibility that drug-reactive IgE an-

tibodies are involved in these adverse reactions was investigated with muscle-relaxant drug-carrier complexes or choline-carrier complexes in radioimmunoassays with patients' sera.⁵⁻¹⁰ Cross-reactivity between muscle relaxants was suggested by skin tests^{2,11} and demonstrated by RAST-inhibition studies.⁷ It appeared that the serum IgE binding sites of the drugs were the ammonium ion determinants,⁷ and it was suggested that at least two ammonium groups need to be present on the molecule to induce release of mediators.¹⁰

However, some facts are not yet completely understood. For instance, pancuronium, a muscle relaxant still bearing two ammonium groups, causes fewer anaphylactic reactions than suxamethonium (Fig. 1). On the other hand, most patients with positive skin tests to suxamethonium have negative skin tests to pancuronium. The purpose of this study then was to study the role of the ammonium groups in the trig-

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