

# Anaphylaxis after ingestion of beignets contaminated with *Dermatophagoides farinae*

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*A 48-year-old man was evaluated for anaphylaxis associated with ingestion of beignets prepared from a commercial mix. Microscopic examination of the patient's beignet mix revealed live Dermatophagoides farinae. Another unopened box from the same source was not infested. Skin test results to aeroallergens and foods, including all beignet mix ingredients, were positive only to D. farinae and D. pteronyssinus extracts. Skin prick test results to an infested mix extract (1:5 wt/vol) were also positive, but no reaction was observed with noninfested mix extract. ELISA inhibition studies demonstrated significant inhibition of the patient's serum binding to D. farinae strips by infested mix extract. Parallel inhibition curves were produced by the infested mix extract and a commercial D. farinae extract. Noninfested mix extract showed no inhibition. RAST analysis with beignet mix discs showed significant binding of the patient's serum IgE to infested mix discs but not to noninfested mix discs. RAST inhibition studies revealed more than 86% inhibition of binding of the patient's IgE to infested mix discs by infested mix extract and D. farinae extract. No inhibition was observed with noninfested mix or 5% fetal calf serum-phosphate-buffered saline. We conclude that the allergen to which the patient reacted was most likely D. farinae and that ingestion of D. farinae may cause anaphylaxis in sensitive persons. (J ALLERGY CLIN IMMUNOL 1993;92:846-9.)*

**Key words:** Anaphylaxis, beignets, *D. farinae*, ELISA inhibition, RAST inhibition

A wide variety of foods have been associated with allergic reactions. In addition to the foods themselves, food contaminants have also been found to be responsible for some IgE-mediated hypersensitivity reactions. In most instances the food contaminant has been another food, which was introduced when inadequately cleaned equipment was used to process more than one food.<sup>1</sup>

In this report we describe a patient in whom anaphylaxis developed shortly after ingestion of homemade beignets prepared from a commercial mix. During an investigation of the patient's food, we found that the beignet mix was contaminated with *Dermatophagoides farinae*. We were able to demonstrate that the patient was highly sensitive to dust mites and not sensitive to food allergens, leading us to conclude that the patient's anaphy-

## Abbreviations used

PBS: Phosphate-buffered saline  
FCS-PBS: Fetal calf serum - phosphate-buffered saline

lactic episode was the result of ingesting dust mites.

## CASE REPORT

A 48-year-old man came into the emergency room complaining of shortness of breath, chest tightness, and a swelling sensation in this throat. He also complained of generalized pruritus, nausea, and a flushed feeling. Before arrival in the emergency room, he had experienced abdominal cramping and two episodes of loose stools. In the emergency room he appeared to be uncomfortable and in mild respiratory distress. Physical examination was remarkable for angioedema of the anterior cervical region, end expiratory wheezes in all lung fields, and diffuse erythema and urticaria. Treatment given included 0.3 ml of 1:1000 subcutaneous epinephrine, 75 mg oral diphenhydramine, and 40 mg of prednisone. Symptoms gradually subsided over 90 minutes.

On questioning, the patient revealed that the reaction had started 15 minutes after he ate four home-

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made beignets. The beignet mix had been purchased at a restaurant in New Orleans, Louisiana, where the patient had eaten beignets without difficulty. The box of beignet mix had been purchased 3 to 4 weeks previously, and it had remained unopened until the day of the reaction.

## METHODS

### Sample preparation

The remainder of the beignet mix was brought to the clinic by the patient. Examination of the mix under light microscopy revealed actively crawling mites in an average concentration of 1 to 2 mites per low power ( $10\times$ ) field. A 1:5 wt/vol dilution of the mite-infested beignet mix was made by adding 2 gm of the mix to 10 ml of phosphate-buffered saline (PBS) without azide. After overnight mixing, the extract was separated by centrifugation. The supernatant was then sterile-filtered into sealed glass bottles.

An unopened box of beignet mix was obtained from the New Orleans establishment. Microscopic examination revealed no live or dead mites. A 1:5 wt/vol extract was made of this noninfested mix.

Serum samples from the patient and from allergic and nonallergic control subjects were obtained and frozen at 20° C until assayed.

### ELISA and ELISA inhibitions

High-binding FB strips (ICN Biomedicals, Horsham, Pa.) were coupled with *D. farinae* extract (Hollister-Stier Laboratories, Spokane, Wash.). The *D. farinae* extract (10,000 allergic units/ml) was diluted 1:50 in a 60 mmol/L carbonate-bicarbonate coupling buffer, pH 7.0, and 50  $\mu$ l of the extract were added per well. After overnight incubation at 4° C, the wells were washed with PBS containing 0.1% Tween 20. The strips were blocked with 300  $\mu$ l per well of 5% fetal calf serum-phosphate-buffered saline (FCS-PBS) for a minimum of 6 hours.

The patient's serum, positive control sera, and negative control sera were assayed by ELISA for *D. farinae*-specific IgE. The procedure, as previously described by Rowe,<sup>2</sup> was modified by use of a chromogenic substrate instead of the fluorogenic substrate. All samples were assayed in triplicate, and results were expressed as the percent mean absorbance of patient wells compared with wells containing a negative serum pool.

The ELISA inhibition assays were performed by adding twofold dilutions of the mite-infested mix, noninfested mix, and *D. farinae* extracts (Hollister-Stier) to equal volumes of the patient's serum overnight at 4° C. Controls consisted of equal volumes of patient serum and 5% FCS-PBS. The final dilutions of the extracts were chosen to provide inhibition values between 20% and 80%. The patient serum-inhibitor mixtures were transferred to the *D. farinae*-coated strips and assayed for mite-specific IgE by ELISA. All dilutions were run

in duplicate, and duplicates were averaged. The percentage of inhibition was calculated by subtracting the mean absorbance of the wells with inhibitor divided by the mean absorbance of the wells without inhibitor from 100%.

### RAST and RAST inhibitions

RAST was performed by the double overnight method as described in the Phadebas RAST procedure (Pharmacia Diagnostics AB, Uppsala, Sweden). The procedure was modified by using 50  $\mu$ l of patient or control serum plus 450  $\mu$ l of Pharmacia diluent per tube. *D. farinae* discs (Pharmacia ENI Diagnostics, Piscataway, N.J.) were used. Samples were assayed in duplicate. Results are expressed as the percent iodine 125-labeled anti-IgE bound, which was obtained by dividing the mean counts per minute of the test sample by the total counts per minute of the <sup>125</sup>I-labeled anti-IgE added, multiplied by 100. A percent binding of greater than three times that of a negative serum pool was considered positive.

Next, discs for RAST analysis were prepared with the beignet mix extracts. Briefly, the mite-infested mix extract and the noninfested mix extract were diluted in 1:10 in 0.1 mol/L borate saline solution, pH 9.0. Two milliliters of the diluted extract were each mixed overnight with 25 cyanogen bromide-activated paper discs in capped glass vials. One milliliter of 1 mol/L ethanolamine was added per vial and mixed for 1 hour. The discs were then washed 10 times with 0.005 mol/L bicarbonate saline solution and stored at 4° C in 10% dextran saline solution until use.

RAST, with the beignet mix discs, was performed according to the modified double overnight procedure as described previously. Results are again reported as percent <sup>125</sup>I-labeled anti-IgE bound.

RAST inhibitions were determined by preincubation of patient and control sera with dilutions of inhibitors or 5% FCS-PBS overnight, before the modified double overnight RAST was performed. A serum pool known to be strongly reactive to *D. farinae* was used as a positive control. Three individual sera without significant reactivity to *D. farinae* were used as negative controls. The chosen dilutions of the inhibitors were based on the ELISA inhibition results. Results are expressed as the percent inhibition of radio-labeled anti-IgE bound as compared with a control reaction in which 5% FCS-PBS was added instead of inhibitor.

### Skin testing

Skin prick and intradermal tests with our usual panel of aeroallergen and food extracts (Hollister-Stier) were performed. In addition, skin prick tests were performed with the prepared infested and noninfested mix extracts. Reactions were considered positive if a wheal at least 3 mm greater than that produced by the saline control occurred.

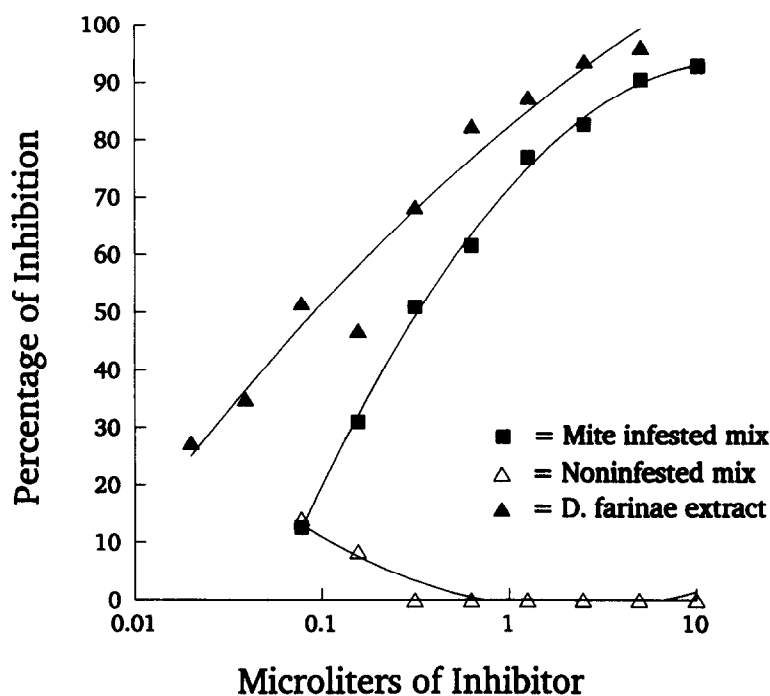


FIG. 1. Inhibition of the binding of the patient's IgE to *D. farinae* by extracts of mite-infested and noninfested beignet mixes and *D. farinae*.

TABLE I. RAST and RAST inhibitions with beignet mixes

| Experiment |                             | Noninfested* | Infested*    |
|------------|-----------------------------|--------------|--------------|
| 1          | Patient serum               | 0.1%         | 1.5%         |
|            | Positive pool               | 0.2          | 1.8          |
| 2          | Patient serum               | 0.07%        | 0.9%         |
|            | Negative 1                  | 0.01         | 0.02         |
|            | Negative 2                  | 0.01         | 0.01         |
|            | Negative 3                  | 0.03         | 0.01         |
| 3          | Patient serum               | 0.07%        | 1.3%         |
|            | + Infested mix              |              | 0.14 (87%)†  |
|            | + Noninfested mix           |              | 1.03 (4.6%)† |
|            | + <i>D. Farinae</i> extract |              | 0.12 (89%)†  |
|            | + 5% FCS-PBS                |              | 1.08         |

\*Results are expressed as percent of total counts bound. More than 0.5% binding was considered evidence of specific IgE.

Noninfested and infested refer to the extracts coupled to the paper discs used for the assays.

†Percent inhibition compared with serum diluted with an equal volume of FCS-PBS.

### Mite identification

A sample of the mite-infested beignet mix was sent to Larry G. Arlian, PhD (Wright State University, Dayton, Ohio). Approximately two dozen adult mites were removed from the mix and identified. All were found to be *D. farinae*.

### RESULTS

The patient underwent skin prick and intradermal testing with commercial extracts to a panel of foods and common aeroallergens. All results were

negative with the exception of significant positive skin prick test reactions to *D. farinae* and *D. pteronyssinus* mites. All of the individual ingredients listed on the beignet mix box were included in the food extracts. During skin prick testing with 1:5 dilutions of the beignet mix extracts, a positive reaction to the infested mix extract and a negative reaction to the noninfested mix extract occurred.

ELISA inhibition results demonstrating inhibition of the patient's serum binding to *D. farinae* are shown in Fig. 1. The inhibition curves pro-

duced by the *D. farinae* extract and the mite-infested beignet mix are parallel. The infested mix demonstrated significant inhibition, whereas the noninfested mix showed no significant inhibition at any dilution.

RAST analysis with *D. farinae* discs showed strong patient reactivity. The patient's serum bound 17.3% of total counts, compared with 12.7% for the positive serum pool and 0.3% for negative serum.

RAST and RAST inhibition results with the beignet mix discs are shown in Table I. In the first experiment the patient's serum and the mite-positive serum pool both displayed positive binding to the infested mix discs but not to the noninfested mix discs. In the second experiment the patient serum again displayed positive binding, but three negative control sera showed no significant binding to either disc. In the third experiment inhibition of binding of the patient's IgE to the infested mix discs occurred with the infested mix and *D. farinae* extracts (87% and 89%, respectively) but did not occur with the noninfested mix extract or 5% FCS-PBS.

## DISCUSSION

In this report we present a 48-year-old man who experienced an anaphylactic episode after ingestion of homemade beignets. Skin prick and intradermal testing to a panel of inhalants and foods revealed significant positive reactions to only *D. farinae* and *D. pteronyssinus* extracts. Skin prick test results to the individual ingredients of the beignet mix were negative, as was the result of a skin test with an extract of the noninfested mix. The patient, however, did react to an extract of the infested beignet mix. The patient declined challenge with beignets made from noninfested mix, but he continues to eat foods with similar ingredients without difficulty. Parallel ELISA in-

hibition curves between a commercial *D. farinae* extract and the mite-infested beignet mix extract demonstrate that the beignet mix allergen to which the patient responded was most likely *D. farinae*. Visual mite identification, RAST, and RAST inhibition assay results support this conclusion.

In industrialized societies a wide variety of additives and contaminants that provoke adverse reactions are found in processed foods.<sup>3</sup> Mold contaminants are prominent in fermented food products such as cheese, dried fruits, yogurt, and wine. Insect parts can contaminate spices and other foods. Drugs, dyes, additives, bacteria, and bacterial products occasionally contaminate foods.<sup>4</sup> The complexity of modern food processing and preparation also provides a means for contamination of one food by another. Recently,<sup>1</sup> adverse reactions were reported to "nondairy foods" in six milk-allergic children. In every case cow's milk protein was implicated as the causative agent.

Thus allergic reactions to foods containing substances not mentioned on the food labels have been previously reported. *D. farinae* sensitivity has been commonly linked to asthma and allergic rhinitis, but to our knowledge, this is the first report of anaphylaxis resulting from ingestion of food contaminated by *D. farinae*.

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