

## Characterization of allergens from spores of the oyster mushroom, *Pleurotus ostreatus*

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*Crude extracts of Pleurotus ostreatus spores obtained from a single local source were fractionated by gel filtration to resolve the allergenic components. The fraction pool corresponding to 10.5 to 25 kd molecular weight contained allergenic activity as demonstrated by both RAST and skin testing. Similar results were obtained with extracts from spores that originated in four other areas and with extracts prepared from P. sajor-caju spores obtained from commercially produced caps. The RAST-active fraction was further separated by hydrophobic interaction chromatography (HIC). HIC fraction pools were assayed for allergen(s) by RAST inhibition and immunoblotting of isoelectric focused polyacrylamide gels. RAST-inhibition data indicated that the allergen(s) was reversibly bound to the HIC column, eluting with 2, 1, and 0.15 mol/L of buffered salt solutions. After electrofocusing, these fractions yielded 15, 12, and 11 Coomassie brilliant blue-staining bands, respectively. IgE binding occurred with 7, 8, and 6 of these bands, as revealed by radiostaining of the immunoblots. These procedures help identify P. ostreatus spore allergens and allow a greater degree of standardization in the preparation of allergen extracts from basidiospores for use in diagnosis and therapy of fungal allergy. (J ALLERGY CLIN IMMUNOL 1988;82:978-86.)*

The role of basidiospores in production of respiratory allergic diseases is at present incompletely characterized. The subdivision Basidiomycotina (basidiomycetes) of the fungal kingdom contains those species of fungi with a basidium, which is a specialized cell in which karyogamy and meiosis occur and which typically produces four basidiospores. In most species, the basidiospores are particularly well suited for dispersal by air and are produced in abundance. Basidiomycetes are the most morphologically complex fungi, including the familiar mushrooms and puff-

### Abbreviations used

HIC: Hydrophobic interaction chromatography  
kd: Kilodalton  
UV: Ultraviolet

balls, plant rusts and smuts, the less obvious jelly fungi, and the wood-destroying "house fungus" and tree "conks."

Basidiospores are prevalent among biologic particles recovered in air samplers.<sup>1-4</sup> Furthermore, basidiospores are routinely collected in air samplers operated in a variety of environments.<sup>1-4</sup> Most of these spores are <10  $\mu$ m in diameter; this is a size range that favors particle retention in the *small airways*.

In contrast to basidiomycetes, the allergenic properties of several species of Deuteromycotina (i.e., Fungi Imperfecti) have been more thoroughly studied. Particular attention has been directed to species of *Alternaria*, *Aspergillus*, and *Cladosporium*, among others.<sup>5-7</sup> Most deuteromycetes are asexual forms of Ascomycotina (ascomycetes).<sup>8,9</sup> Ascomycetes, and by extension, deuteromycetes, are closely related to the Basidiomycotina and together comprise the "higher fungi."<sup>8</sup> Airborne spore dispersal, which is used extensively by both groups of fungi, places large

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numbers of spores in the atmosphere and therefore in potential contact with respiratory tract mucosa.

In a prior study,<sup>10</sup> 32% of patients with either allergic rhinitis or asthma demonstrated positive reactions by prick skin test to one or more basidiospore extract. Many of these subjects also had elevated levels of serum IgE antibodies to basidiospores.<sup>11</sup> Other studies have demonstrated allergenicity of basidiospore extracts by intradermal<sup>12</sup> and prick<sup>13</sup> skin testing and by skin testing and bronchial provocation tests.<sup>14</sup> The allergenicity of basidiomycete culture extracts has also been reported.<sup>15</sup> These results, when they are considered with the abundance and ubiquity of basidiospores in the air, suggest that airborne basidiospores are important in respiratory allergy.

The present study was undertaken to purify and characterize the allergenic component(s) of *Pleurotus ostreatus* (Jacq. ex Fr.) Kummer. This species was chosen because it is locally plentiful, it occurs widely throughout North America and Europe, it produces abundant spores, a reliable spore extraction method has been devised, and a reasonable number of available patients were skin test and RAST positive.

## MATERIAL AND METHODS

### Patient population and skin testing

Volunteers with a personal history of respiratory atopic disease were skin prick tested with 12 common inhalant allergens (Greer Laboratories, Lenoir, N.C. or Hollister-Stier Laboratories, Elkhart, Ind.) and *P. ostreatus* to confirm the presence of allergy. *P. ostreatus* skin test reagents were prepared by resuspending lyophilized material (extracts or gel filtration pools) in phosphate-buffered saline. These solutions were passed through a 0.45 µm filter and then were diluted 1:1 with sterile glycerol to final concentrations of 10, 1, 0.1, and 0.01 mg/ml. Results were recorded at 20 minutes and were considered positive if the mean wheal diameter was 2 mm greater than the negative control (phosphate-buffered saline). Histamine diphosphate at 1 mg/ml was used as the positive control to demonstrate skin reactivity. Sera were obtained from skin test-reactive patients for RAST and for immunoblot analysis.

### Spore collection and extraction

Basidiocarps (mushroom caps) were collected fresh in the New Orleans area and brought to the laboratory. Caps were placed, gills down, on sheets of Whatman No. 1 filter (Whatman, Inc., Clifton, N.J.) paper, covered with additional sheets with the edges secured with tape, and left overnight to cast spores. Spore prints thus obtained were scraped from the filter paper. *P. sajor-caju* spores were a gift from Dr. William Reinhardt of the Campbell Soup Co. All spores were essentially pure (>95%), as determined by light microscopy. Spores were defatted with ether (200 ml/gm of spores) before extraction. Defatted spores were allowed to air-dry overnight and then were homogenized in

0.125 mol/L of  $\text{NH}_4\text{HCO}_3$  buffer, pH 8.1, for 2 minutes in a Braun homogenizer (B. Braun Melsungen AG, Melsungen, West Germany) cooled with escaping liquid  $\text{CO}_2$ . The homogenized suspension was centrifuged for 30 minutes at 80,000 g, and the supernatant was lyophilized. Lyophilized crude extract was stored under desiccation until it was used.

### Chromatography

Lyophilized crude spore extract was resuspended in 0.125 mol/L of  $\text{NH}_4\text{HCO}_3$  at approximately 200 mg/ml. The solution was centrifuged at 8000 g for 5 minutes, and the supernatant was applied to a Sephadex G-75 column 5 by 75 cm equilibrated with the same buffer at 4° C. Ten milliliter fractions were collected dropwise, and UV absorbance was determined at 280 nm. Fractions were pooled and lyophilized according to regions of the UV absorbance scan.

Gel filtration fractions demonstrating RAST and skin test activity were further resolved by HIC with a phenyl-Sepharose column (Pharmacia Diagnostics, Piscataway, N.J.). Samples were applied in 0.05 mol/L of Tris buffer, pH 7.5, with 4 mol/L of NaCl. A stepwise elution was used, including 4, 2, 1, and 0.15 mol/L of NaCl in 0.05 mol/L of Tris, followed by  $\text{H}_2\text{O}$  and 20% (vol:vol) ethylene glycol. Fractions thus obtained were dialyzed exhaustively against 0.05 mol/L of  $\text{NH}_4\text{HCO}_3$  and lyophilized.

### RAST and RAST inhibition

Cellulose disks activated with cyanogen bromide were coupled with lyophilized extracts or gel filtration pools resuspended in borate buffer at 5 mg/ml. A serum pool from five skin test-positive individuals was used at 100 µl per disk. After an overnight incubation, unreacted serum was removed with three 2 ml washes of 0.09% NaCl (saline). A total of 25,000 counts of  $^{125}\text{I}$ -labeled equine antihuman IgE (Kallested Laboratories, Austin, Texas) was added per disk and incubated overnight, and then disks were washed three times with saline. Residual isotope activity was determined by accumulating counts for 5 minutes in a model 5500 gamma counter (Beckman Instruments, Inc., Irvine, Calif.). All assays were performed in duplicate. Results were calculated as the percent of the total counts added that was bound to the experimental disks. Lyophilized RAST-active fraction pool from gel filtration was resuspended at 5 mg/ml in borate buffer and coupled to disks for RAST inhibition. RASTs were inhibited by incubating disks with 100 µl of a 1:1 mixture of the serum pool and the test solution (i.e., HIC pools).

### Isoelectric focusing and immunoblotting

Protein samples were focused in thin-layer polyacrylamide gels (5% T/3% C) following manufacturers recommendations for power limits and total time (Pagplates, LKB, Bromma, Sweden). Portions of gels to be stained were fixed before staining by immersion for 1 hour in a solution of 3.5% (wt:vol) sulfosalicylic acid and 11.5% (wt:vol) trichloroacetic acid. Fixed gels were equilibrated in staining solvent (3:1:9, methanol:acetic acid:water) for 20 minutes

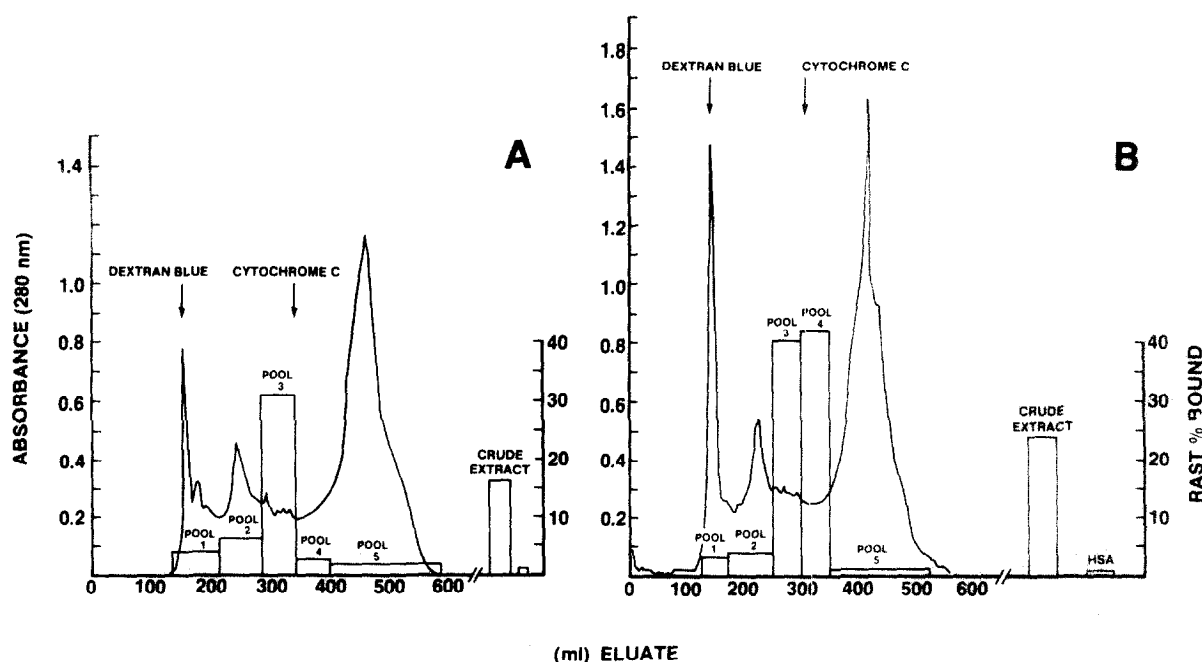


**FIG. 1.** UV absorbance (280 nm) patterns for crude extracts. **A**, *P. sajor-caju* and **B**, *P. ostreatus* spore extracts after gel filtration in Sephadex G-75 (scale at left). Five milliliter fractions were collected, pooled as indicated, lyophilized, resuspended, and used to coat RAST disks. RAST assays used a pool of five skin test-positive sera. Histograms indicate RAST reactivity (scale at right).

and then stained in 0.1% (wt:vol) Coomassie brilliant blue R-250 for 20 minutes in staining solvent initially at 60° C. Gels were destained with washes of staining solvent.

Focused proteins were blotted by passive transfer to 0.2  $\mu$ m pore size nitrocellulose membranes that had been activated with cyanogen bromide according to the method of Demeulemester et al.<sup>16</sup> After blotting, the nitrocellulose membranes were removed from the gel by soaking in 50% glycerol in 0.1 mol/L of phosphate buffer, pH 7.5. Stock-blocking buffer was prepared with 0.2 mol/L of phosphate buffer (pH 7.5) with 2% Tween 20 and 5% bovine serum albumin. This stock-blocking buffer was diluted 1:5 with ion-depleted water for a 1-hour incubation of the blotted nitrocellulose after removal from the gel. Stock-blocking buffer (diluted 1:2 with water) was used to dilute serum and radiolabeled anti-IgE to working strength. Washes after each step were with phosphate buffer with 0.5% Tween 20.

The portion of the nitrocellulose membrane corresponding to each lane of the gel was divided lengthwise into two strips. One of each pair of strips was incubated with a serum pool from skin test-positive and RAST-positive individuals. The other strip of each pair was incubated in a serum pool from individuals skin test and RAST negative to *P. ostreatus* but demonstrating allergic reactions to other common inhalants (house dust, animal danders, or pollens). The positive pool and negative pool contained 900 and 650 IU/ml total IgE, respectively. IgE-binding bands were detected by subsequent incubation with <sup>125</sup>I-labeled (250,000 cpm per

strip) anti-IgE (Kallested Laboratories) overnight, removing unbound label, and exposing on x-ray film in the absence of light at -80° C for 48 hours.

## RESULTS

Gel filtration of the crude spore extracts resolved three major peaks of UV absorbance for both *P. sajor-caju* (Fig. 1, A) and *P. ostreatus* (Fig. 1, B). For further analysis, fractions from each peak, as well as fractions corresponding to the region between peaks 2 and 3 were combined into pools as indicated. RAST with pooled sera of skin test-positive individuals and lyophilized material from the five gel filtration pools indicated that the area between peaks 2 and 3 contained the highest levels of IgE-binding activity (Fig. 1). The fractions comprising these pools corresponded to a molecular weight range of 10.5 to 25 kd.

RAST analysis after gel filtration was repeated with spore extracts obtained from five different areas within the city of New Orleans to assess the degree of variability of source material (data not presented). A further comparison was made with extract of spores from commercially produced *P. sajor-caju*. Although some minor differences were observed in the UV absorbance patterns, the RAST data indicated that the al-

**TABLE I.** RAST results from nine individual sera tested against *P. ostreatus* crude spore extract and five gel filtration fractions

| Patient   | Crude extract | Antigen tested         |     |      |     |     |
|-----------|---------------|------------------------|-----|------|-----|-----|
|           |               | Sephadex G-75 fraction |     |      |     |     |
|           |               | 1                      | 2   | 3    | 4   | 5   |
| H. V.     | 6.6*          | 1.4                    | 2.8 | 24.8 | 1.8 | 1.2 |
| D. E.     | 6.3           | 4.6                    | 3.4 | 25.2 | 2.0 | 1.3 |
| S. T.     | 1.6           | 1.0                    | 1.5 | 4.8  | 0.9 | 0.9 |
| M. A.     | 4.8           | 0.9                    | 1.1 | 21.7 | 1.9 | 0.8 |
| T. I.     | 14.4          | 26.2                   | 7.9 | 5.4  | 3.2 | 1.9 |
| G. O.     | 1.6           | 0.9                    | 1.0 | 6.3  | 1.0 | 0.9 |
| P. O.     | 3.0           | 1.3                    | 1.5 | 7.5  | 1.8 | 1.3 |
| N. O.     | 14.0          | 1.1                    | 2.5 | 23.5 | 2.8 | 1.0 |
| W. I.     | 6.0           | 2.0                    | 2.6 | 16.2 | 1.6 | 0.9 |
| $\bar{X}$ | 6.5           | 4.4                    | 2.7 | 15.0 | 1.9 | 1.1 |

\*Values are the percent of total label added that bound to the solid phase; < 3% bound was considered a negative RAST assay.

**TABLE II.** Prick skin test results

| Level of response* | Crude extract | Antigen tested         |   |   |   |   |
|--------------------|---------------|------------------------|---|---|---|---|
|                    |               | Sephadex G-75 fraction |   |   |   |   |
|                    |               | 1                      | 2 | 3 | 4 | 5 |
| 0.1                |               |                        |   | 2 |   |   |
| 1                  | 5†            | 1                      | 1 | 6 | 1 |   |
| 10                 | 3             | 2                      | 4 |   | 2 |   |
| Negative           |               | 5                      | 3 |   | 5 | 8 |

\*Response level is the lowest concentration (milligram per milliliter) of extract that elicited a positive reaction.

†Positive reactions among eight patients who were tested with crude *P. ostreatus* spore extract and five fraction pools obtained from gel filtrations.

lergenic activity was present in the same fraction pool in all sources with activity.

To establish whether or not RAST detects all fungal allergens, Sephadex G-75 column pools were assayed further by RAST (Table I) and skin prick testing (Table II) in nine individuals skin test positive to *Pleurotus* spore extracts. Skin tests indicated that the pools with high RAST activity also induced positive skin prick tests (Table II). Although the data on skin testing are summarized in Table II, when the skin test data were examined individually by subject, RAST values for the different fractions corresponded to skin test reactivity for that fraction.

In one case, skin test reactivity occurred with the fractions from the first two peaks at 1 mg/ml. Serum from this patient (T. I.) also elicited positive RAST values with these two pools, especially pool 1. This patient had atopic dermatitis that might have affected

allergenic reactivity because of high overall IgE levels.

The lyophilized pool of Sephadex G-75 allergen activity was further fractionated with a phenyl-Sepharose column (Fig. 2). Most of the material in the Sephadex G-75 fraction failed to bind to the column and eluted with the 4 mol/L of NaCl. This material consistently demonstrated very low RAST-inhibition activity (Fig. 3). However, material that eluted with the 2, 1, and 0.15 mol/L of NaCl buffers demonstrated significant RAST-inhibition activity. When the column was stripped with ethylene glycol, very little material was recovered, and that material contained minimal RAST-inhibition activity.

Isoelectric focusing of the Sephadex G-75 allergen fractionated by HIC resolved a number of bands staining with Coomassie brilliant blue (Fig. 4). The starting material (Sephadex G-75 pool) yielded eight

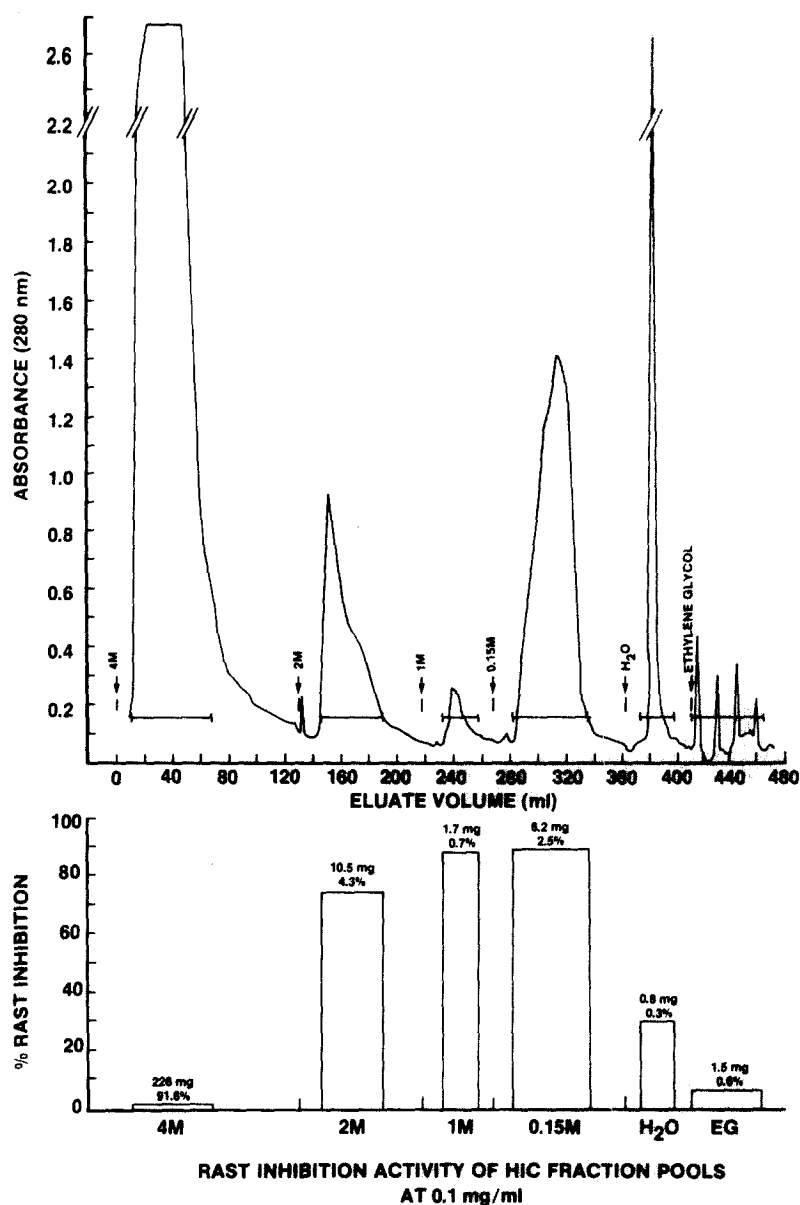


**FIG. 2.** UV absorbance (**top**) of fractions recovered after a stepwise elution from phenyl-Sepharose of the gel filtration pool with RAST activity. Gel filtration pool with RAST activity was lyophilized, resuspended, and loaded onto the column. Position of *arrows* indicates the eluate volume at which the buffer steps began. Fractions were pooled as indicated by *horizontal bars*. RAST-inhibition activity (**bottom**) of each pool from the phenyl-Sepharose column was determined. RAST disks were coated with gel filtration material that was loaded onto the phenyl-Sepharose column. *Histogram bars* are aligned beneath the corresponding column pool. *Numbers* above each bar refer to the milligram amount recovered from each pool and the percent of total material recovered.

bands, the 4 mol/L fraction yielded nine bands, 2 mol/L yielded 15 bands, 1 mol/L yielded 12 bands, 0.15 mol/L yielded 11 bands, H<sub>2</sub>O yielded one band, and no bands were observed in the ethylene glycol fraction.

IgE-binding bands were observed by autoradiography in the immunoblot procedure. The Sephadex

G-75 fraction had seven bands that bound IgE, the 2 mol/L fraction had seven, 1 mol/L had eight, and the 0.15 mol/L of HIC fraction had six bands that bound IgE (Fig. 5). The 4 mol/L H<sub>2</sub>O and ethylene glycol fractions had only faint, poorly resolved radiostaining in the high pH portion of the immunoblot.

The radiostaining bands exhibited a wide range of

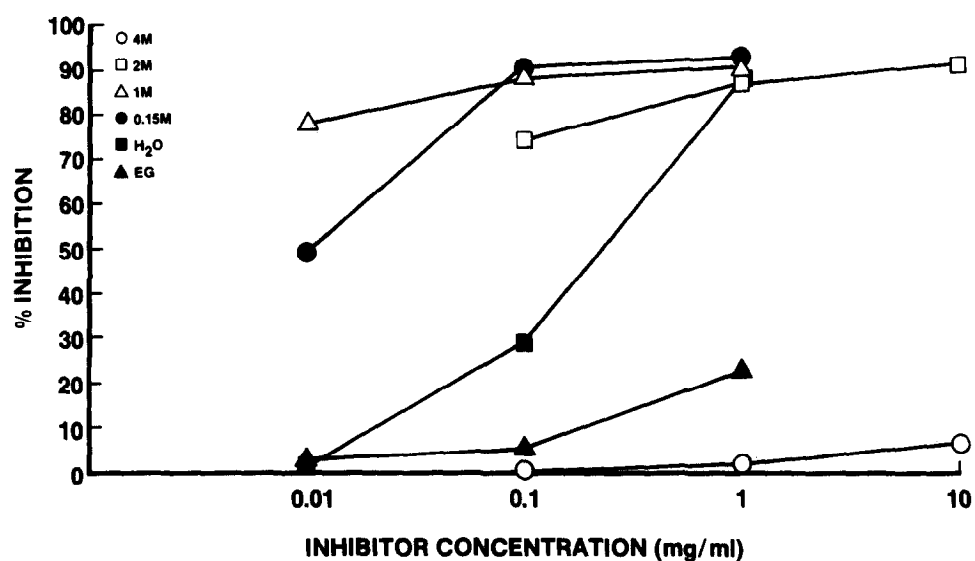


**FIG. 3.** Inhibition of RAST with HIC fractions. RAST disks were coated with RAST-active gel filtration pool material, and the RAST reaction was inhibited with the various phenyl-Sepharose pools at three concentrations.

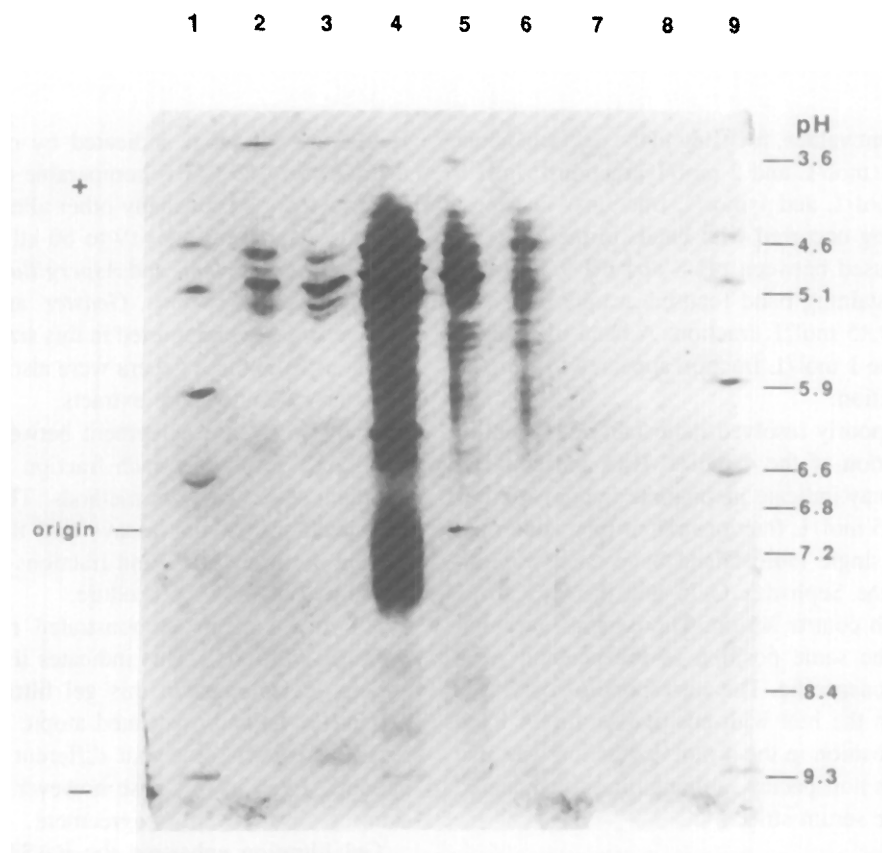


**FIG. 4.** Isoelectric focusing gel stained with Coomassie brilliant blue. *Lanes 1 and 9*, isoelectric point markers corresponding to the scale at *right*; *lane 2*, starting material (gel filtration pool); *lane 3*, 4 mol/L phenyl-Sepharose pool; *lane 4*, 2 mol/L; *lane 5*, 1 mol/L; *lane 6*, 0.15 mol/L; *lane 7*, H<sub>2</sub>O; *lane 8*, ethylene glycol. All lanes were loaded with 40  $\mu$ l of resuspended extract at 20 mg/ml. A replicate set of lanes from the same gel was used to blot proteins with nitrocellulose.

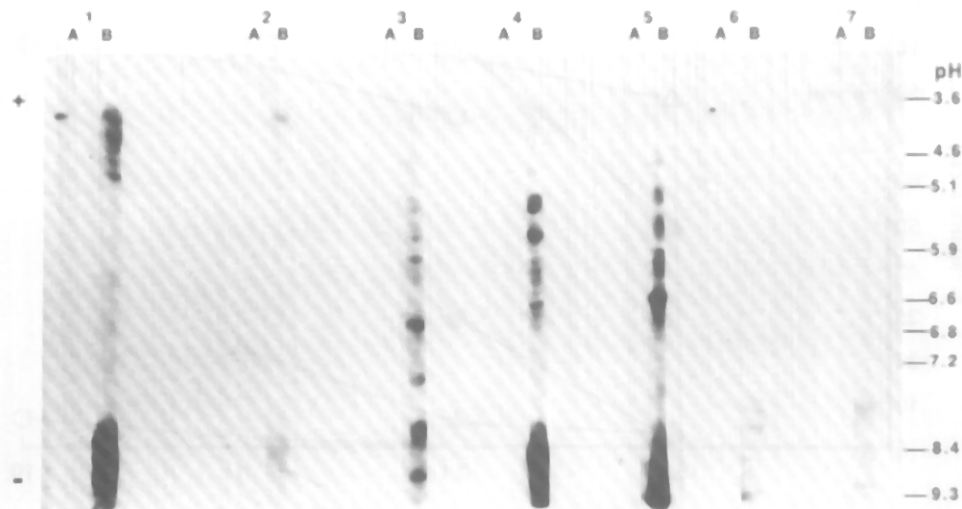


**FIG. 5.** Autoradiograph of samples electrofocused as in Fig. 3 and immunoblotted by passive transfer to 0.2  $\mu$ m pore size nitrocellulose that had been activated with cyanogen bromide. Focused proteins from the starting material (Sephadex G-75 RAST-active pool) were blotted onto nitrocellulose strip 1; proteins from 4 mol/L of gel lane onto strip 2; 2 mol/L lane onto strip 3; 1 mol/L lane onto strip 4; 0.15 mol/L lane onto strip 5; H<sub>2</sub>O lane onto strip 6; and the ethylene glycol lane onto strip 7. Each blotted strip was divided lengthwise into two pieces. One piece (A) was incubated in a pool of skin test negative and one piece (B) in a pool of skin test-positive, atopic sera.

isoelectric point values, focusing in the gel from about pH 4 in the 1 mol/L and 2 mol/L fractions to pH 9 in the 0.15 mol/L and 1 mol/L fractions. The most intense staining occurred with bands in the basic region that focused between pH 8 and pH 9. Another intense radiostaining band focused at approximately pH 6 in the 0.15 mol/L fraction. A band of reduced intensity in the 1 mol/L fraction appeared to focus at the same position.

The faint, poorly resolved radiostaining evident in the basic portion of the 4 mol/L H<sub>2</sub>O and ethylene glycol blots may indicate incomplete fractionation of the 1 and 0.15 mol/L fractions rather than additional allergens. A single radiostained band each was observed with the Sephadex G-75 and the H<sub>2</sub>O strips incubated with control serum. These bands appeared to focus at the same position in the gel and were regarded as nonspecific. The corresponding band was disregarded in the blot with positive serum. A band at the same position in the 4 mol/L fraction was also disregarded as nonspecific, although it only appeared in the positive serum strip.

## DISCUSSION

Fractionation of crude spore extracts by gel filtration provided data on the molecular weight range of the allergenic component(s) of *P. ostreatus*. The mo-

lecular weight range indicated by our gel filtration data (10.5 to 25 kd) is comparable to the molecular weights reported for many other allergens, including those of deuteromycetes (9 to 60 kd), such as *Alternaria*, *Cladosporium*, and *Aspergillus*.<sup>6</sup> Basidiospore allergens from *Coprinus*, *Geaster*, and *Calvatia* also have recently been reported in this size range.<sup>17,18</sup> The allergens from these genera were also obtained by gel filtration of crude spore extracts.

There was close agreement between the skin test and RAST results for each fraction for the eight individuals tested by both methods. This demonstrates the specificity of our assays and that all allergens present in our extracts (and fractions thereof) are detected by our RAST procedure.

All nine patients demonstrated reactivity to the same fraction pool. This indicates that the major allergens are retained in this gel filtration pool. One patient (T. I.) had associated atopic dermatitis and a reaction pattern somewhat different from the other patients. Even in this case however, the RAST and skin test results were in agreement.

Gel filtration enhanced the RAST activity of the active pool relative to crude extract. This could result from either a greater concentration of allergen(s) in the Sephadex G-75 fraction, by removal of inert material, or the removal of a substance(s) inhibitory to

the coupling reaction between allergens and RAST disks.\* The crude extract was RAST positive, which argues against the presence of inhibitors. We believe that the increased activity is due to greater concentration of allergen in the gel filtration pool from which extraneous material has been removed. The homogenization procedure used in preparing these extracts completely disrupts the spores, releasing most if not all soluble material, including many presumably extraneous compounds. Fractionation of these extracts is thus warranted, since more material is released into solution by homogenization than by gentle incubation of spores in buffer. Fractionation also yields a more active gel filtration pool that is less likely to yield false negative results.

It is well established that fungal allergens have a high degree of variability concerning source material; this subsequently impedes research efforts.<sup>5-7, 19</sup> In the present study, however, the comparison of spore extract fractionation by gel filtration indicated allergenic similarities. These similarities were consistent among the sources of *P. ostreatus* spores from basidiocarps growing in different areas of the city and with the commercially produced *P. sajor-caju*.

Liengswangwong et al.<sup>20</sup> also compared different sources of *P. ostreatus* spore extracts in their study by RAST and high-performance liquid chromatography. The UV absorbance patterns after high-performance liquid chromatography of the different extracts were generally similar, but with some specific differences. RAST values of extracts from different sources were comparable, however. Their results in conjunction with the present study indicate that various local sources do not differ greatly regarding allergenic properties.

Subsequent fractionation via HIC of the RAST-active Sephadex G-75 pool indicated that the allergen(s) present had some degree of hydrophobicity, since they were retained by a phenyl-Sepharose column. This method of chromatography proved particularly useful because approximately 89% by dry weight of the material recovered from the Sephadex G-75 RAST-active pool passed through the column with 4 mol/L of NaCl and contained no discernable activity in RAST inhibition. This allows removal of a large amount of apparently extraneous material in a single step.

Coomassie brilliant blue staining of electrofocused gels revealed that, even after both Sephadex G-75 and HIC column chromatography, *P. ostreatus* allergen

fractions contained from 9 to 13 bands. This indicates that further separations are needed to obtain pure allergens. Immunoprints demonstrated that among the three fractions with activity, at least seven components were able to bind IgE from the serum of allergic patients (Fig. 5). Some of these bands could, however, be degradation products or isomers of allergens.<sup>21</sup> The immunoprints of the electric focused HIC fractions (Fig. 5) indicated that most of the IgE-binding activity was in the 2, 1, and 0.15 mol/L fractions that agrees with the RAST-inhibition data.

Previous reports of allergenic activity of basidio-spores have been based on the use of crude extracts. The material recovered from our fractionations had enhanced activity both in the RAST and skin test. This demonstrates the importance of fractionating crude extracts prepared by homogenizing spores. This also suggests that, if fractionated extracts are used, the prevalence of skin test-reactive patients may be greater than previously indicated. The use of fractionated extracts, due to their enhanced allergenic activity, therefore appears warranted for both in vitro serologic techniques and for further testing of patient populations.

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## Double-blind, placebo-controlled food challenge (DBPCFC) as an office procedure: A manual

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*There is now enough experience with the use of double-blind, placebo-controlled, food challenge (DBPCFC) to recommend its use as an office procedure for most patients complaining of adverse reactions to foods. This manual discusses the practical methods required for the allergist to undertake DBPCFC in the office. Thorough histories supplemented by food allergen skin testing are used to design a DBPCFC that carefully attempts to reproduce the history of food-induced symptoms described by the patient. Precautions that must be taken are delineated before challenge, as is treatment that may be required if a reaction occurs. For those foods to which challenges are positive, longitudinal evaluation with repeated challenge at appropriate intervals help to determine whether or not the problem will resolve over a period of time. (J ALLERGY CLIN IMMUNOL 1988;82:986-97.)*

The purpose of this article is to share the authors' experience using DBPCFC in outpatient settings. As such, it was designed to outline the stepwise procedures useful in evaluating the validity of a history of

### Abbreviation used

DBPCFC: Double-blind, placebo-controlled food challenge

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adverse reactions to foods. The procedures as presented here have been endorsed in principle by the Adverse Reactions to Foods Committee of the American Academy of Allergy and Immunology.

The DBPCFC is the state-of-the-art technique to confirm or refute histories of adverse reactions to foods.<sup>1-10</sup> Although this is the "gold standard" by which all studies of food allergy should be judged, it is also a practical procedure by which practicing al-