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Antigenic and allergenic analysis of psyllium seed components

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*The outer portions (husk) of psyllium seeds are a concentrated source of natural fiber used in some bulk-fiber laxatives and cereals. They are known to elicit respiratory allergic reactions after inhalation or ingestion among sensitized individuals. Antigenic and allergenic characterization of three psyllium-seed fractions (husk, endosperm, and embryo) was conducted with crossed immunoelectrophoresis (CIE), crossed radioimmuno-electrophoresis, and sodium dodecyl sulfate-polyacrylamide gel electrophoresis to determine the source of psyllium allergenicity. Homologous CIE demonstrated psyllium endosperm and embryo extracts contained seven and four antigens, respectively. Husk extracts were too gelatinous to react by CIE. However, heterologous CIE profiles of endosperm or embryo extracts, reacted with antihusk antibodies, resulted in antigen-antibody precipitin peaks that matched the heavy staining precipitin lines of homologous reactions for endosperm and embryo, respectively. These results indicated that commercial-grade husk, endosperm, and embryo contained similar antigens. Extracts of all three seed components contained antigens that bound IgE antibodies in the sera of 11 psyllium RAST-positive individuals, as determined by crossed radioimmuno-electrophoresis. The few prominent husk protein/peptide bands resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis were common in either embryo or endosperm. Immunoblots revealed common IgE reactive bands in all three seed fractions. Microscopic examination of the powdered commercial-grade psyllium (95% pure) revealed it contained endosperm and embryo particles. These immunologic, biochemical, and microscopic findings suggest that other contaminating seed components are primarily responsible for the allergenicity of commercial-grade psyllium-husk powder rather than the husk itself. (*J ALLERGY CLIN IMMUNOL* 1992;89:866-76.)*

Key words: Psyllium, psyllium husk, allergenicity, laxatives, cereals, fiber

The outer coat (husk) of psyllium seeds (*Plantago ovata*) is a concentrated source of natural soluble fiber used in some bulk-fiber laxatives and cereals because

Abbreviations used

CIE: Crossed immunoelectrophoresis
CIE: Crossed radioimmuno-electrophoresis
SDS-PAGE: Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
BSA: Bovine serum albumin

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of its laxative properties and, recently, its demonstrated effect in reducing serum cholesterol.¹⁻⁴ This fiber has the potential for eliciting respiratory allergic reactions in some sensitive individuals after inhalation

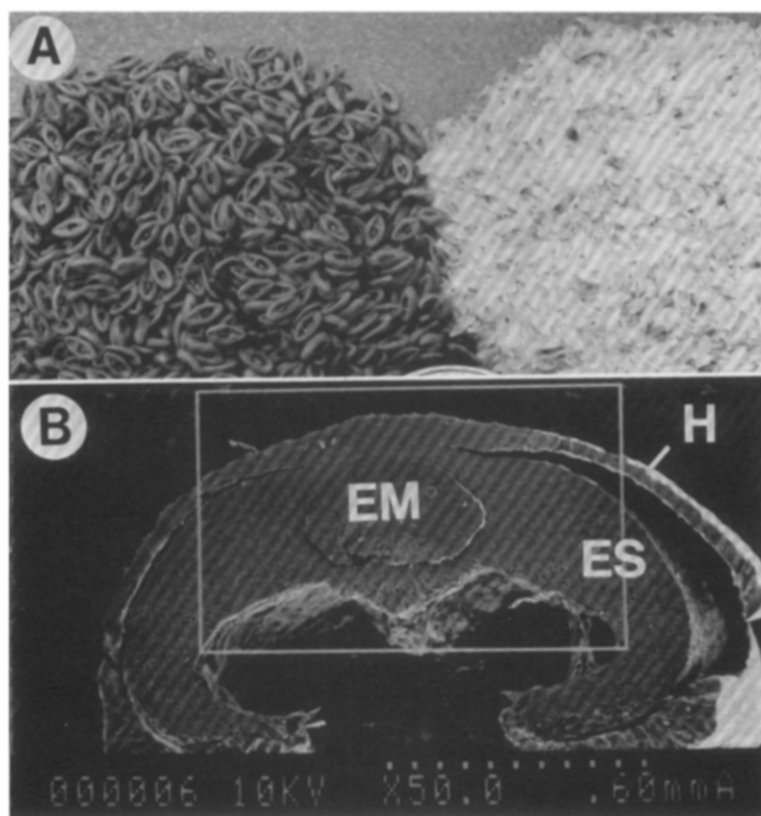


FIG. 1. Components of psyllium seed, *Plantago ovata*. **A**, Whole seeds (*left*) and commercial husk (*right*). **B**, Cross section of seed revealing endosperm (*ES*), embryo (*EM*), and husk (*H*).

or ingestion. Sensitization is most likely to occur after repeated inhalation exposure to psyllium dust in certain occupational settings.⁵⁻¹⁹ The specific source of the allergenicity has been unclear. Antigenicity could originate directly from the husk itself and/or from contaminating seed components remaining after husk has been separated from the seed.

Endosperm makes up the bulk of the psyllium seed (Fig. 1). An elongate brownish embryo is located within the endosperm and extends lengthwise in the seed. The endosperm is surrounded by a single layer of transparent mucilaginous epidermal cells that is termed the husk or seed coat. The three components generally separate when the seeds are crushed. The transparent husk is separated from the other seed components, pulverized, and used in the laxative because of its powerful hygroscopic properties.

Previous investigators reported no evidence of protein in husk preparations and suggested that the psyllium allergy was due to a gum composed of a mixture of polysaccharides.^{7, 8, 20, 21} In this study we examined the antigens and allergens of a commercial grade of psyllium husk (95% purity) and two seed fractions (endosperm and embryo) by CIE, CRIE, SDS-PAGE, immunoblotting, and microimmunodiffusion to char-

acterize and determine the source of psyllium allergenicity.

MATERIAL AND METHODS

Human sera

Eleven sera from individuals that were RAST (Pharmacia Diagnostics, Piscataway, N.J.) positive (class II or greater) to psyllium-husk powder were used to perform CRIE and immunoblotting. Of the eleven sera, three were class V, four were class IV, two were class III, and two sera were class II. Ten of these eleven RAST-positive individuals also exhibited clinical symptoms of allergy associated with exposure to psyllium. Typical symptoms described included runny nose, itchy eyes, and shortness of breath. Three RAST-negative individuals (class 0) served as control subjects (Table I). Most individuals worked in a bulk-laxative manufacturing plant.

Extract preparations

To prepare endosperm and embryo extracts, dehusked psyllium seeds or embryos were milled (ball and Wiley mills with 20 to 30 mesh) and added to enough 0.1 mol/L of phosphate-buffered saline to make 1:20 or 1:5 (wt/vol) mixtures for endosperm or embryo extracts, respectively. Mixtures were extracted overnight (shaking at 21° C) and centrifuged, and the supernatants were removed and frozen (-20° C). The protein contents of the endosperm and em-

TABLE I. Relative IgE binding to psyllium-seed components by psyllium-sensitive individuals.

Serum	RAST cpm (class)	CRIE		
		Psyllium embryo (homologous)	Psyllium endosperm (homologous)	Endosperm (Ag)/husk (Ab) (heterologous)
1	13,354 (IV)	+++	+++	+++
2	3,137 (II)	+	+	+-
3	14,613 (IV)	+	++	++
4	19,567 (V)	+++	++++	+++
5	13,407 (IV)	++++	++++	++++
6	6,945 (III)	+++	+++	++
7	18,907 (V)	++++	++	++
8	20,786 (V)	++++	++++	++++
9	5,160 (III)	+++	+++	++
10	13,133 (IV)	+++	+++	++
11	2,737 (II)	+	++	+-
12*	228 (0)	—	—	—
13*	286 (0)	—	—	—
14*	292 (0)	—	—	—

Ag, Antigen; Ab, antibody; —, no IgE binding, autoradiogram blank after 10 days; +-, very weak IgE binding, very light radiostaining after 10 days; +, weak IgE binding, light radiostaining after 10 days; ++, moderate IgE binding, moderate radiostaining after 5 days; +++, strong IgE binding, dark radiostaining after 5 days; + + + +, very strong IgE binding, very dark radiostaining after 5 days.

*Sera from control individuals that were not psyllium sensitive.

bryo extracts, as determined by Kjeldahl's analysis, were 3.8 and 21 mg/ml, respectively. Commercial-grade dry psyllium-husk material (Indian 95% psyllium husk) was added to glass-distilled water to make a 1:20 (wt/vol) mixture, stirred, and allowed to extract (4° C) overnight. The gelled mixture was then cold centrifuged, and the supernatants (extract) were used for studies within 1 hour of centrifugation. The protein content of the extract of Indian 95% psyllium husk was determined by Bio-Rad protein assay (Bio-Rad Laboratories, Richmond, Calif.) and was 0.06 mg/ml. In addition, extract was prepared from a small amount of commercial psyllium husk that was further purified by the differential-density Freon flotation process. The purified psyllium husk was 99% or more pure. Purified husk, commercial-grade husk, powdered endosperm, and embryo contained 0.6%, 2.7%, 24%, and 41.2% protein via Kjeldahl's method of determining nitrogen, respectively.

In an attempt to free the protein from the carbohydrate portion of the psyllium endosperm, cellulase was added to the extract used for the heterologous CIE and CRIE reactions of endosperm antigen and antihusk serum. One milligram of cellulase (Sigma Chemical Co., St. Louis, Mo.) was added per 200 µl of psyllium endosperm extract. It was incubated 2 hours at 36° C and centrifuged, and the supernatant was used within 2 hours.

Rabbit antisera

Antisera to commercial-grade husk, endosperm, and embryo were produced in New Zealand white rabbits. For initial immunizations, 0.5 ml emulsions of extracts in 0.9% NaCl and Freund's complete adjuvant (Calbiochem Corp., La Jolla, Calif.) (1, 1, and 0.1 mg/ml of protein for endosperm,

embryo, and husk, respectively) were injected subcutaneously into two nuchal sites per rabbit. One milliliter intraperitoneal booster injections were administered to each rabbit for 4 consecutive days beginning 31 days after the initial injections. Rabbits were exsanguinated 45 days after the initial injections. All sera were individually analyzed by microimmunodiffusion and CIE, and high-titer sera were pooled and frozen.

Microimmunodiffusion assays

Microimmunodiffusion was performed on pattern B immunoplates (Hyland Laboratories, Costa Mesa, Calif.) as previously described.²² Wells were 2.5 mm in diameter and consisted of five peripheral wells 7 mm from a central well. Five microliters of antiserum and extract were used for each reaction.

CIE and CRIE

The numbers of antigens and allergens present in commercial-grade husk, endosperm, and embryo extracts were determined by CIE and CRIE, as previously described.²²⁻²⁵ Ten to 25 µl of psyllium embryo or endosperm supernatant extract were horizontally electrophoresed in the first dimension with Svendsen's buffer* (pH 8.7) at 200 V for 30 minutes. Second dimensions were electrophoresed at 50 V for 14 to 18 hours. Anodal gels contained 75 to 180 µl of rabbit antiserum per 3 ml of agarose, whereas cathodal gels contained 17 to 60 µl of rabbit antiserum per milliliter. Attempts to electrophorese a husk antigenic extract were

*Svendsen's buffer: 89.6 gm of diethyl barbaturic acid, 177.2 gm of Tris, 2.1 gm of calcium lactate, and 4 L of sodium azide.

unsuccessful because of its high viscosity. However, a rabbit antiserum made to commercial-grade husk was used in heterologous CIE and CRIE gels.

For tandem CIE, two separate wells were punched 4 mm apart in the first dimensional agarose gel.²² Extracts were placed into the wells and allowed to diffuse for 30 minutes, and then the wells were filled with agarose. The gels were electrophoresed in the first and second dimension as for regular CIE gels.

Identification of allergens was done by CRIE as described previously.^{22, 26, 27} CIE gels were incubated overnight (room temperature) in 0.75 to 1 ml of human serum per 19 ml of RAST A buffer, washed three times in RAST B buffer, and incubated (room temperature) in ¹²⁵I-labeled antihuman IgE (Kallestad Diagnostics, Inc., Chaska, Minn.) (0.5 to 1 million cpm per 20 ml of RAST C buffer). The compositions of RAST A, B, and C buffers were as previously described.^{26, 27} Gels were washed, dried, and placed in cassettes with tungsten-enhancement screens and Kodak (Eastman Kodak, Rochester, N.Y.) X-Omat RP film at -80°C for varying lengths of time. To visualize light and dark peaks, autoradiograms of the different components were exposed for varying combinations of 1, 2, 3, 4, 5, 7, 8, 10, 11, 14, 15, 17, 24, 26, and/or 28 days, depending on radiostaining intensity. The intensity of radiostaining on the precipitin lines was used to semiquantitate IgE binding to particular allergens. To standardize and to make comparisons both within and between sera, all gels were processed identically in one batch with one batch (lot) of reagents.

SDS—PAGE

Extracts of commercial-grade and purified psyllium husk, endosperm, and embryo were separated by SDS—PAGE with a discontinuous buffer system, as previously described.²⁸ The 12% polyacrylamide separating gel contained 0.1% SDS in 0.375 mol/L of Tris HCl, pH 8.8, and the stacking gel contained 5% acrylamide and 0.1% SDS in 0.125 mol/L of Tris HCl, pH 6.8. The running buffer contained 0.025 mol/L of Tris, 0.19 mol/L of glycine, and 0.1% SDS, pH 8.3. Ten to 40 µl of the seed component extracts in equal volumes of sample buffer (0.125 mol/L of Tris, 4% SDS, 20% glycerol, and 0.002% bromophenol blue, pH 6.8) and 2 µl of 2-mercaptoethanol were electrophoresed vertically at 140 V (constant voltage) until the bromophenol blue marker reached the bottom of the gel. A mixture of four protein standards (BSA, 66 kd; egg albumin, 44 kd; trypsinogen, 24 kd; and lysozyme 14.3 kd) were also electrophoresed to estimate the molecular weights of the protein/peptide bands. Reference gels were stained with silver nitrate.

Blot transfer and autoradiography

Proteins and peptides were transferred from the SDS—PAGE gels to nitrocellulose membranes with a Bio-Rad Trans-Blot cell system. The unstained SDS gels were placed against an equilibrated (soaked 30 minutes in transfer buffer) nitrocellulose membrane between two filter paper sheets. The sandwich was placed between fiber pads, secured in a

rigid, hinged holder, and placed into the Trans-Blot cell. Proteins and peptides were transferred anodically (toward the anode) at 280 mA (constant current) for 2¼ hours. The transfer buffer (Blitz buffer) contained 50 mmol/L of Tris, 192 mmol/L of glycine, 0.1% of SDS, and 20% of methanol, pH 8.3. The SDS—PAGE gel was stained with silver nitrate to verify the transfer. Also, one lane on the nitrocellulose membrane was removed and stained (amido black) to confirm the transfer. The remaining nitrocellulose membrane was incubated overnight in 8 mol/L of urea, 0.1 mol/L of 2-mercaptoethanol, and 5 mg/ml of BSA to block the binding sites, then incubated overnight in 50 mmol/L of Tris HCl, and dried. Nitrocellulose strips were individually incubated in human sera (0.75 to 1 ml/19 ml of RAST A buffer) and then in ¹²⁵I-labeled antihuman IgE with the same conditions as for the CRIE gels. The nitrocellulose strips were washed three times with Tris HCl after the last RAST B wash instead of the gel washing and pressing. The dried strips were placed into the cassettes, and autoradiography was performed.

RESULTS

Microimmunodiffusion

Reaction of psyllium endosperm extract with rabbit antiendosperm serum resulted in four precipitin lines. Similar reaction of embryo extract with rabbit antiembryo serum revealed two precipitin lines. Likewise, cross-reaction of endosperm extract with an antihusk serum resulted in one precipitin line. When antiendosperm serum was interposed between antihusk-containing wells, one dense and one diffuse precipitate formed. The dense husk precipitate was completely fused with the endosperm precipitate.

CIE

CIE reaction of endosperm extract with rabbit antiendosperm serum revealed six different anode-migrating antigens and one cathode-migrating antigen (Fig. 2, A and B). Four anode-migrating antigens were identified when an embryo extract was reacted with rabbit antiembryo serum (Fig. 2, C and D). One antigen/antibody precipitin peak, resulting from homologous CIE reactions of both the embryo (No. 3) and endosperm (No. 1), revealed prominent staining with Coomassie blue. Other antigenic precipitates were less well defined with staining restricted to the legs of the precipitin peaks.

Antigens in embryo and endosperm extracts demonstrated similar electrophoretic mobility (precipitin peak position) and staining properties (antigen/antibody precipitates). This finding suggested that the different seed fractions contained common antigens. If this were the case, then the commercial-grade husk extract might also contain these common antigens. Since husk extract preparations were too viscous to

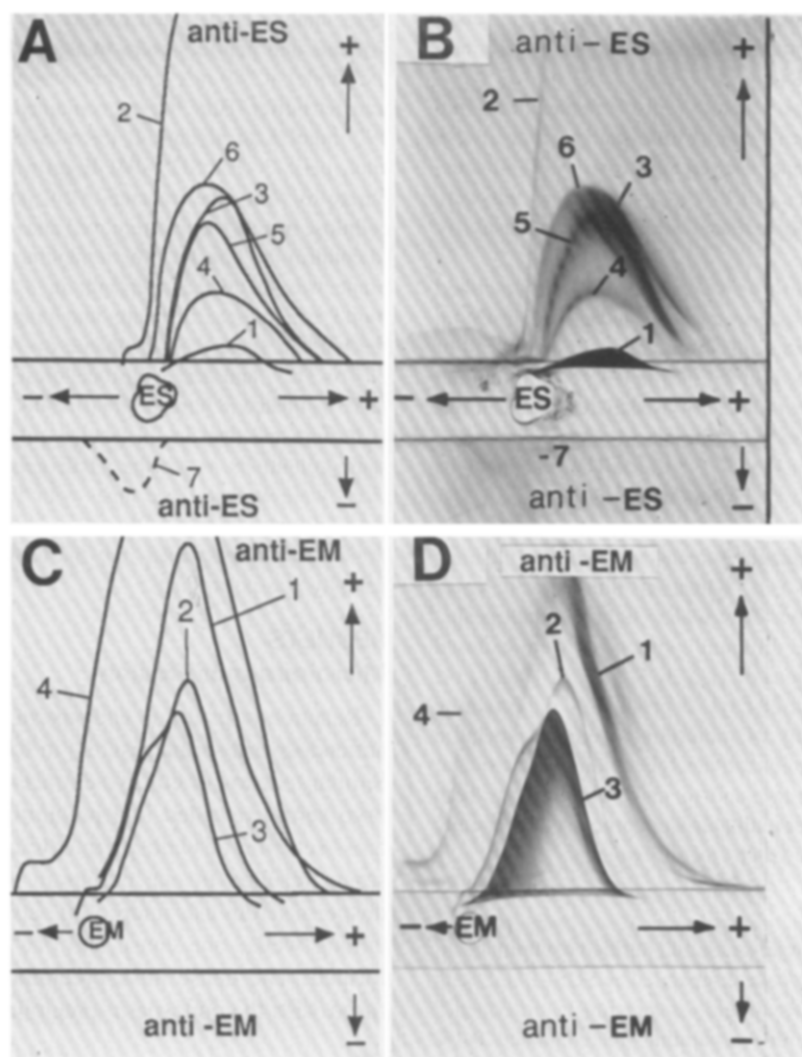


FIG. 2. Psyllium endosperm (ES) extract reacted with rabbit anti-ES serum. **A**, Schematic profile. **B**, Homologous CIE pattern. Psyllium embryo (EM) extract reacted with rabbit anti-EM serum. **C**, Schematic profile. **D**, Homologous CIE pattern.

use in the CIE technique, heterologous CIE reactions were performed to determine this possibility. CIE reactions of endosperm or embryo extracts with antihusk serum (Fig. 3, A and B) revealed heavy Coomassie blue-staining precipitates that matched the heavy staining precipitate lines of homologous reactions for endosperm and embryo, respectively (Fig. 2, A and B). Heterologous reactions of endosperm with embryo and embryo with endosperm (Fig. 3, C and D) also revealed similar precipitates. This provides additional evidence of the existence of common antigens. Position, size, and staining properties of the common peaks were identical, suggesting that endosperm, embryo, and husk each contained the same antigens but with varying quantities, as indicated by the differing peak heights. To confirm these results, several tandem reactions were performed.

Tandem

In one set of tandem CIE experiments, endosperm and embryo extracts were placed in separate tandem wells and then reacted with either antiendosperm or antiembryo serum. Both tandem reactions resulted in identical precipitin peak profiles. These reactions resulted in the fusion of the short and tall prominently staining peaks. It was not possible to evaluate whether other peaks were fused because they were either diffuse or extended off the gels.

In a similar control set of tandem experiments, either endosperm or embryo extract was placed in both separate tandem wells and reacted with their respective antiserum. In each case, the prominently staining antigen/antibody peaks were fused as expected. Embryo or endosperm extract placed in separate tandem wells and reacted with antihusk serum revealed fused

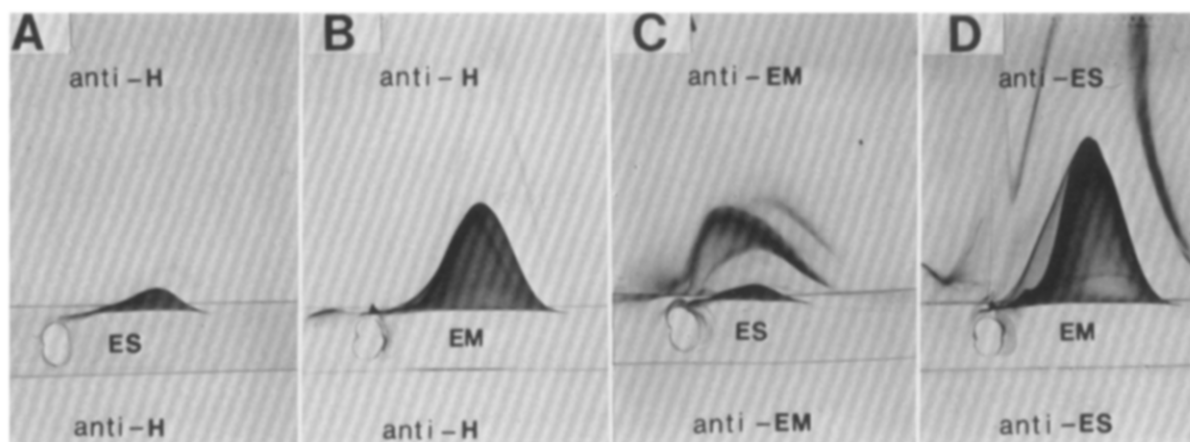


FIG. 3. Heterologous CIE patterns. **A**, Psyllium endosperm (*ES*) extract reacted with rabbit anti-husk (*H*) serum. **B**, Psyllium embryo (*EM*) extract reacted with rabbit anti-*H* serum. **C**, Psyllium *ES* extract reacted with rabbit anti-*EM* serum. **D**, Psyllium *EM* extract reacted with rabbit anti-*ES* serum.

precipitin peaks (Fig. 4) identical to the homologous tandem embryo or endosperm reaction. Weaker Coomassie blue-staining peaks for all tandem reactions were not sufficiently visible to clearly discern if they were also fused.

CRIE

Autoradiographs of CIE plates in which endosperm extract was reacted with antiendosperm serum demonstrated that at least four negatively charged and one positively charged antigen bound IgE in the sera of 11 RAST-positive individuals (Fig. 5). Eight individuals demonstrated strong IgE binding to the same two antigens (Nos. 1 and 3). Three of these eight individuals demonstrated strong binding to one additional antigen (No. 2). Six individuals demonstrated binding to the electropositive antigen (No. 7). Likewise, autoradiography of CIE reactions of endosperm extract with antihusk serum were made (Fig. 6). The prominent short peak, which corresponded to a peak in the homologous endosperm reaction, and the tall faint peak demonstrated radiostaining. One additional antigen that only appeared when the endosperm extract was treated with cellulase also demonstrated IgE binding.

Similarly, all four antigens that were identified in embryo extract bound IgE when antigens reacted with the 11 sera. Autoradiographs for two representative sera are illustrated in Fig. 7. All 11 sera demonstrated binding to antigen Nos. 1, 2, and 3. Four sera contained IgE directed at antigen No. 4.

Intensity of radiostaining (concentration of IgE in the sera) correlated well with RAST scores that were obtained with a husk extract (Table I). All non-

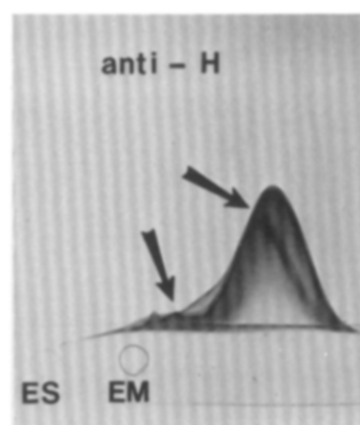


FIG. 4. Tandem CIE patterns of psyllium endosperm (*ES*) and embryo (*EM*) extracts in separate wells reacted with rabbit antiserum built to husk (*H*). Arrows indicate fused short *ES*/anti-*H* (left) and tall *EM*/anti-*H* (right) peaks, respectively.

sensitive control subjects demonstrated no IgE binding/radiostaining.

SDS-PAGE

SDS-PAGE resolved the commercial-grade husk, endosperm, and embryo extracts into many protein/peptide bands over a wide molecular weight range between 10 and 66 kD (Fig. 8). There were several corresponding bands in the three extracts. Commercial husk extract had bands between 14 and 24 kD, between 24 and 44 kD, and around 44 kD that corresponded with bands of endosperm and embryo extracts.

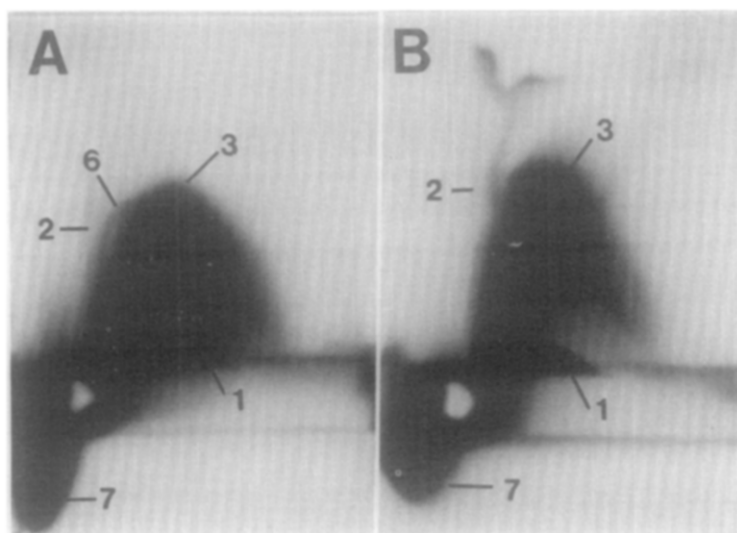


FIG. 5. Homologous CRIE IgE-binding pattern of psyllium endosperm extract reacted with rabbit antiendosperm serum. **A** and **B**, Representative autoradiograms with reference sera 9 and 6, respectively, as listed in Table I. Corresponding CIE pattern illustrated in Fig. 2, *B*.

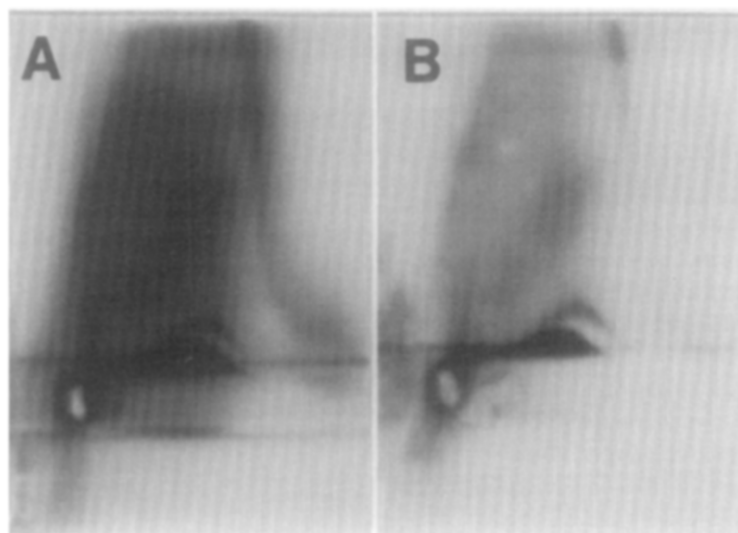


FIG. 6. Heterologous CRIE IgE-binding pattern of psyllium endosperm extract reacted with rabbit anti-husk serum. **A** and **B**, Autoradiograms with representative sera 5 and 8, respectively, as listed in Table I. Corresponding CIE pattern illustrated in Fig. 3, *A*.

There were also distinct quantitative and qualitative differences in the extracts. Embryo and endosperm contained some common bands that were not present in husk; however, all heavy silver-staining bands of commercial husk were common in either embryo or endosperm. Generally, commercial husk contained fewer prominent (dark staining) bands than either embryo or endosperm. Purified husk extracts exhibited

only a few weakly staining bands in comparison with commercial husk extract.

Protein/peptide bands of commercial husk, endosperm, and embryo were transferred to nitrocellulose, and IgE bound bands were detected after incubation in patients' sera and autoradiography. All three extracts exhibited many IgE reactive bands in the 10 to 66 kd range (Fig. 9). Most CRIE-positive individuals

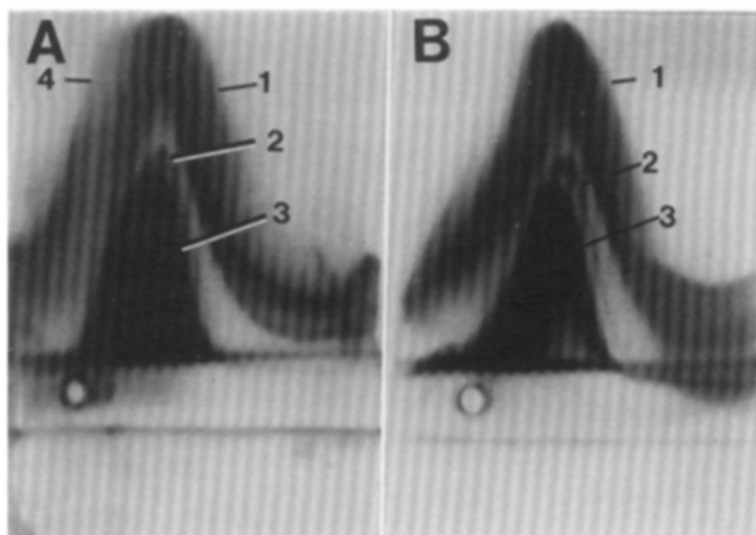


FIG. 7. Homologous CRIE IgE-binding pattern of psyllium embryo extract reacted with rabbit antiembryo serum. **A** and **B**, Autoradiograms with representative sera 4 and 6, respectively, as listed in Table I. Corresponding CIE pattern illustrated in Fig. 2, *D*.

had IgE antibody to bands of all three extracts. Controls consisting of sera from RAST-negative individuals demonstrated no binding.

DISCUSSION

The antigenic and allergic components of three psyllium seed components were investigated. The results of this study clearly demonstrate that extracts prepared from commercial-grade psyllium husk, endosperm, and embryo seed components contain proteins that bind IgE antibodies. Commercial psyllium husk that is used in laxatives and cereals is available in varying grades of purity up to 95% pure. It appears that the allergens in commercial psyllium husk and, thus, in laxatives and cereals, come from the more protein-rich seed components, such as the embryo and endosperm.

Commercial-grade husk powder swells when it is soaked in water and forms a transparent mucilage. When it is sufficiently concentrated, this gel was too viscous to use in CIE techniques. However, in this study, this preparation was used to immunize rabbits, and its antigens and allergens were then evaluated indirectly with heterologous CIE/CRIE and cross-reactions with the other seed components. Supernatants from diluted husk preparations could be fractionated on SDS-PAGE, and the allergens were assayed by immunoblot and autoradiography.

Homologous CIE demonstrated that embryo and endosperm contained at least four and seven antigens,

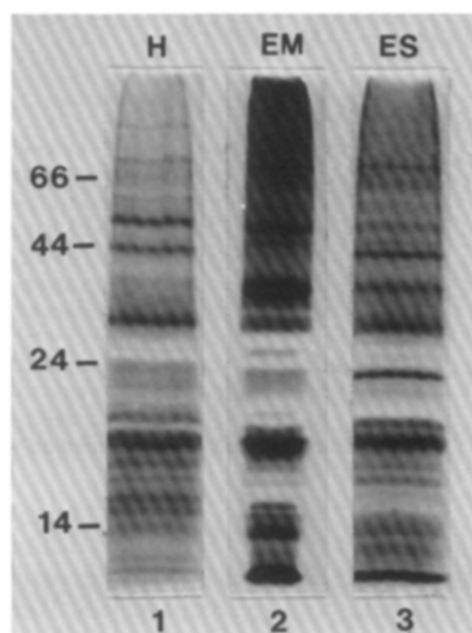


FIG. 8. SDS-PAGE fractionation profile. Gels were stained with silver nitrate. Molecular weight markers: BSA, (66 kd), egg albumin (44 kd), trypsinogen (24 kd), and lysozyme (14.3 kd); 1, psyllium husk (*H*); 2, psyllium embryo (*EM*); 3, psyllium endosperm (*ES*).

respectively. All four antigens in embryo and at least five of seven antigens in endosperm were allergens and bound IgE in the sera of psyllium-sensitive individuals.

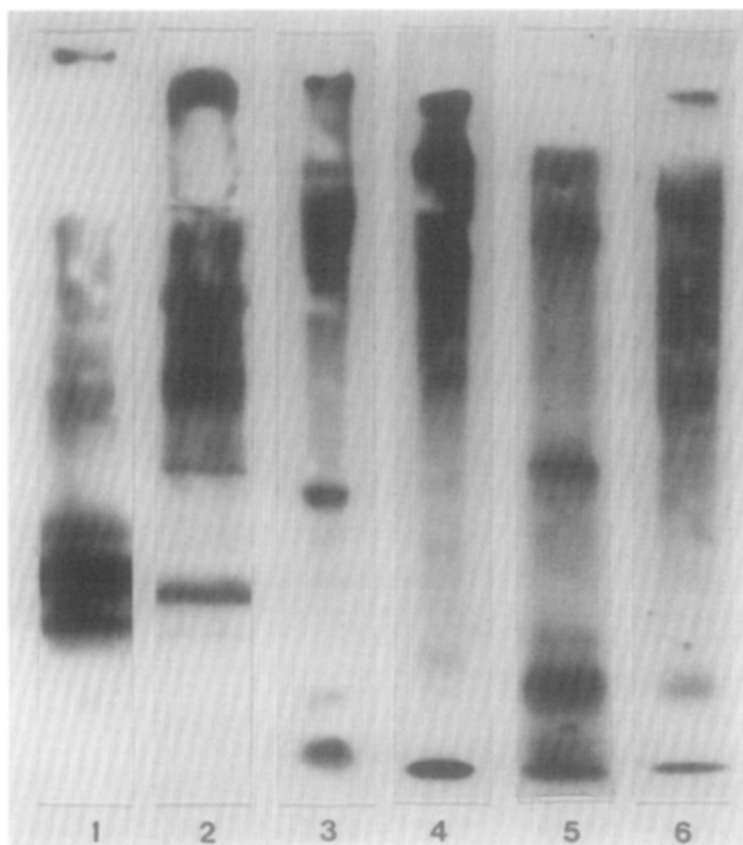


FIG. 9. Immunoblot analyses of antigens in psyllium-seed components recognized by two representative sera. Proteins were separated with SDS-PAGE, transferred electrophoretically to nitrocellulose membranes, incubated in sera of psyllium-sensitive subjects and ^{125}I -labeled antihuman IgE; 1 and 2, autoradiograms of husk allergens recognized by antibodies from representative sera 5 (4-day exposure) and 8 (11-day exposure), respectively, as listed in Table I; 3 and 4, autoradiograms of embryo allergens recognized by antibodies from representative sera 5 (3-day exposure) and 8 (3-day exposure), respectively, as listed in Table I; 5 and 6, autoradiograms of endosperm allergens recognized by antibodies from representative sera 5 (14-day exposure) and 8 (7-day exposure), respectively, as listed in Table I. Only autoradiograms of shorter exposure times are illustrated to visualize better some individual bands. Therefore, not all bands that bound IgE can be observed in this figure. Strong IgE binding bands over exposed entire lane and masked individual bands for longer exposure times.

Single well heterologous CIE with rabbit antiserum to all three psyllium preparations clearly demonstrated that they contained cross-reactive antigens. Heterologous CIE with embryo and endosperm extracts in tandem wells that were reacted with antiendosperm or antiembryo serum demonstrated fusion of the major precipitin peaks. Similar results were obtained when embryo and endosperm in tandem were reacted with antihusk serum. CIE profiles of embryo or endosperm extract that were reacted with antihusk serum elicited precipitin peaks that were similar to reactions of embryo and antiembryo or endosperm and antiendosperm, respectively, for the major staining antigens. This finding, along with similar peak position and Coomassie blue staining, strongly suggested the three

contained similar components. The major difference in CIE profiles for embryo, endosperm, and husk was in peak height, which suggested only differences in the quantities of each antigen between extracts. Based on the immunologic data alone, it was not clear if this cross-reactivity was due to a common entity in each seed component or was due to cross-contamination of the husk raw material with either or both seed components. Microscopic examination of the powdered commercial-grade psyllium husk used in these studies revealed a heterogeneous powder containing both brownish and whitish-colored granules in addition to the transparent husk mucilage. The brownish granules appeared to be pieces of embryo, whereas the white flakes resembled seed endosperm. Additional evi-

dence that commercial husk contains contaminating protein is provided by results of Kjeldahl's nitrogen analysis and SDS-PAGE protein profiles of a purified husk sample. Freon-treated husk (99% or more pure) contained only 0.6% protein compared with 2.7% in the commercial (95% pure)-grade material. SDS-PAGE protein profiles of purified husk extract with negligible seed parts (endosperm and embryo) exhibited very weak silver-stained protein/peptide bands compared with that of the commercial-grade material. Because of the small amount available, the specially purified husk material was not immunoassayed. These physical observations, supported by the immunologic and biochemical findings, strongly suggest that the immunogenicity and allergenicity of bulk psyllium powder used in laxatives originates primarily from the other psyllium seed components that contaminate commercial husk lots.

Previous investigations with SDS-PAGE, immunoblot, protein determinations, and ultraviolet spectroscopy concluded there was no evidence of protein in commercial-grade psyllium used in laxatives.^{7, 8, 21} However, the previous studies used very diluted extracts (1 gm/185 ml) and less sensitive techniques for demonstrating protein than were used in this investigation and that of James et al.²⁹ In contrast, this present investigation demonstrated that the powdered commercial-grade husk contained 2.7% protein. An extract (1:20 wt/vol) made from this commercial husk contained 0.06 mg/ml of soluble protein. Heterologous CIE antigen/antibody precipitates with rabbit antiserum built to this extract and SDS-PAGE profiles with this extract elicited Coomassie blue and silver-stainable protein peaks and bands, respectively. These results confirmed the presence of soluble protein in the commercial-grade psyllium husk.

Some SDS-PAGE resolved protein bands, and CIE antigen/antibody precipitates bound IgE. IgE binding by both assays provides evidence that the allergenicity of psyllium originates from the protein-containing components. This conclusion is consistent with the results of a recent study that concluded that allergic protein associated with the seed husk of psyllium appears responsible for the allergenic reactions to psyllium-containing cereals.²⁹

In conclusion, it appears that psyllium husk, used commercially in bulk laxatives and, recently, in cereal products is comprised of the hydroscopic husk, containing little soluble native protein itself, and some level of contaminating endosperm and embryo seed parts contributing most soluble proteins. Since these unnecessary seed parts appear to be responsible for the allergenic potential of psyllium products, then use of more highly purified husk could reduce the protein

content and thus the allergenicity of products containing psyllium.

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