

Characterization of water-soluble shrimp allergens released during boiling

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Water-soluble shrimp allergens released during boiling (shrimp water) were characterized and compared to allergen extracts from boiled shrimp (shrimp meat). Both shrimp extracts contained acidic proteins (isoelectrofocusing) and demonstrated similar allergenic activity (RAST and RAST inhibition). Shrimp-water extract was analyzed further by immunoprinting with sera from 14 shrimp-sensitive, RAST-positive subjects, and six nonsensitive, RAST-negative individuals. Although none of the sera from shrimp-tolerant individuals reacted, 12/14 sera (85.7%) from shrimp-sensitive subjects reacted with shrimp-water proteins with acid isoelectric points. Shrimp-water extract was fractionated by chromatofocusing with pH and NaCl gradients. A number of eluted ultraviolet-absorbing peaks contained allergens as determined by RAST inhibition. Isoelectrofocusing demonstrated many protein bands present in these peaks, some of which bound IgE from a RAST-positive sera pool. These studies indicate that shrimp water is an excellent source of shrimp allergens, that chromatofocusing is a useful method for fractionation of shrimp allergens, and that shrimp allergens are generally protein molecules with acid isoelectric points. (J ALLERGY CLIN IMMUNOL 1990;85:1005-13.)

In recent times, there has been renewed interest in allergen identification and characterization. This is due in part to the requirements for standardization and quantitation of allergens in commercially available extracts, as well as the need for purified allergens to study further the immunopathogenesis of allergic diseases.^{1, 2} Although allergens from pollens, venoms, and house dust mites have been well characterized, purified allergens from only a few foods, such as cows' milk,³ hens' eggs,³ and cod fish^{4, 5} have been available.³ There have also been studies of allergen identification and/or characterization of com-

Abbreviations used

PBS:	Phosphate-buffered saline
IEF:	Isoelectric focusing
pI:	Isoelectric point
INH-50:	Concentration inhibiting RAST by 50%
UV:	Ultraviolet
PAG:	Polyacrylamide gel

ponents of cereal grain,⁶ soybean,⁷ peanut,⁸ and seafood.⁹⁻¹⁴

Seafood, including crustacea, is frequently identified as inducing adverse reactions in food-sensitive individuals.¹⁵ Crustacea are also important occupational allergens in seafood-processing workers.¹⁶ Previously reported studies of adverse reactions to shrimp in shrimp-sensitive, food-allergic subjects have demonstrated predominantly type I, IgE-mediated, immediate hypersensitivity reactions.^{10, 17, 18} Concurrent studies have investigated allergens present in crustacea, particularly shrimp.⁹⁻¹³ Our present studies, undertaken to extend these findings, demonstrate that the fluid in which shrimp is boiled is an excellent source of shrimp allergens. These allergens can be readily fractionated by chromatofocusing.

MATERIAL AND METHODS

Patients and sera

Fourteen adults with a clinical history of immediate hypersensitivity reactions after the ingestion of shrimp were

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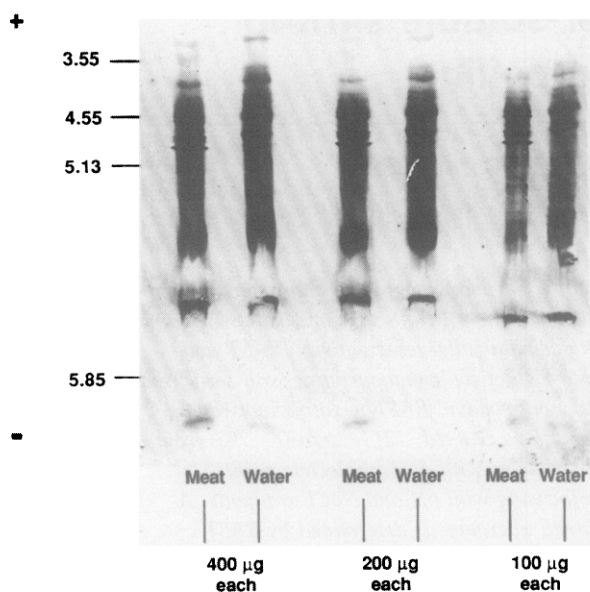


FIG. 1. IEF of shrimp-meat and shrimp-water extracts; 400, 200, and 100 μ g of shrimp meat or water extracts were focused over a pH range of 4.0 to 6.5. Gels were stained with Coomassie brilliant blue.

enrolled in this study. All these subjects were atopic (two or more positive skin test reactions to a panel of 10 common inhalant allergens and a personal history of allergy). Five of the 14 shrimp-sensitive patients were challenged by double-blind, placebo-controlled procedure with shrimp extract, and all five patients had positive responses. Six atopic adults with a negative history of crustacea sensitivity served as control subjects. All subjects gave informed consent, completed a questionnaire concerning their history, and donated a blood sample.

Shrimp extracts

White shrimp (*Penaeus setiferus*), purchased from a local seafood store, were boiled for 15 minutes in ion-depleted water. Both the fluid in which the shrimp was boiled (shrimp water) and the meat of boiled shrimp (shrimp meat) were used as sources of shrimp antigens. The meat of the cooked shrimp was removed and homogenized in PBS, pH 7.2, for 3 minutes in a Waring blender (Waring Products Division, New Hartford, Conn.). After mixing overnight at 4° C on a shaker, extracts were centrifuged (44,000 g), and supernatants were concentrated on an Amicon YM2 filter (Amicon, Danvers, Mass., molecular weight cutoff >1000 daltons). Concentrates were dialyzed (Spectropore 3, Spectrum Medical Industries, Inc., Los Angeles, Calif., molecular weight cutoff >3500 daltons) against PBS and recentrifuged (78,000 g). Dry weight was determined, and then the extract was aliquoted, frozen, and stored at -20° C.

The shrimp water was allowed to cool, sodium azide was added to 0.1%, immediately concentrated on an Amicon YM2 filter (4° C), dialyzed (Spectropore 3) against water at 4° C, and centrifuged (78,000 g). The supernatant was lyophilized and was stored at -20° C.

Total and shrimp-specific IgE levels

Total serum IgE levels of the study subjects were determined by IgE PRIST performed according to manufacturer's instructions (Pharmacia Diagnostics AB, Uppsala, Sweden). Specific serum IgE levels were determined by RAST and performed as previously described.¹⁸ Shrimp antigens in 0.1 mol/L of borate buffer (pH 8.0) at 10 mg/ml were coupled by CNBr-activated paper disks, as described by Ceska and Lundkvist.¹⁹ All tests were performed in duplicate, the mean counts per minute bound to disks was determined, and the results were expressed as percent of total counts per minute added. Significant reactivity was determined to be $\geq 3\%$ label bound, as previously reported.¹⁸

Allergenic activity

Allergenic activities of the two shrimp extracts were compared by RAST inhibition. Briefly, 50 μ l of PBS containing increasing concentrations of test material and 50 μ l of RAST-positive sera pool were simultaneously added to disks coupled with shrimp antigens. The sera pool was composed of three sera with RASTs of 12.5%, 42.3%, and 49.5% label bound to shrimp-water extract. RASTs were performed in duplicate, as described above, means were determined, and results were expressed as percent inhibition of the control RAST (serum with PBS). Positive control sera bound 5500 (shrimp water) to 6250 (shrimp meat) cpm, and negative control sera bound <500 cpm.

IEF and immunoprinting

Thin-layer PAG (5% transmission/3% cross-linkage) (IEF) was performed across the pH range 4.0 to 6.5 according to manufacturer's recommendations for power limits and total time (LKB PAG plates, Bromma, Sweden). Twenty microliters of each test material (20 mg/ml) was applied to each plane of the PAG plate. A lane with pI markers (amyloglucosidase: pI, 3.55; trypsin inhibitor: pI, 4.55; lactoglobulin A: pI, 5.13; and carbonic anhydrase: pI, 5.85, Sigma Chemical Co., St. Louis, Mo.) was included on the part of the gel to be stained with Coomassie brilliant blue.

On completion of IEF, half the gel was removed, and proteins were detected by fixing in a solution of 11.5% trichloroacetic acid and 3.5% sulfosalicylic acid, and staining with Coomassie brilliant blue in 20% methanol/8% acetic acid. The remaining half of the gel containing focused proteins (from boiling water) was blotted by passive transfer to 0.2 μ m pore size nitrocellulose membrane (Hoeffer Scientific Instruments, San Francisco, Calif.). After nitrocellulose membrane was blotted, the membrane was incubated for 1 hour in 0.04 mol/L of phosphate buffer, pH 7.5, with 1.0% bovine serum albumin and 0.4% Tween 20 (blocking buffer) to block unreacted sites. The membrane was washed with PBS plus 1% Tween 20 and then was cut into 5 mm strips. The strips were incubated with appropriate serum samples (RAST positive or negative serum or positive sera pool) and then washed in phosphate buffer/Tween 20 and incubated overnight in ¹²⁵I-labeled equine antihuman IgE diluted with blocking buffer (50,000 cpm per strip). Experimental sera or pooled sera (tested at 1:5 or 1:40) and

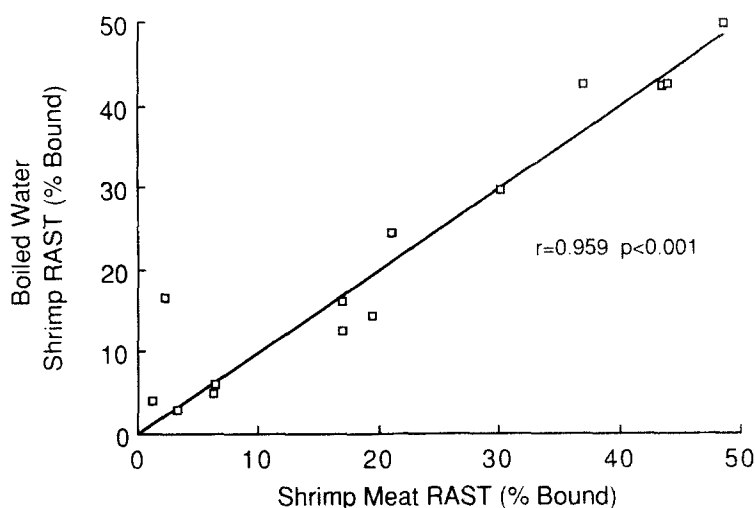


FIG. 2. Comparison of RAST of individual sera to shrimp-meat and shrimp-water extracts. Individual sera were tested for their reactivity by RAST to shrimp meat or water extracts. Results were analyzed by linear regression, and the r value of 0.959 was highly significant ($p < 0.001$).

control sera (tested at 1:2) were diluted to contain similar quantities of total IgE. Unbound anti-IgE was removed by washing, and autoradiography was performed on x-ray film at -70°C for 48 hours.

Chromatofocusing

Chromatofocusing was performed (PEE-94, Pharmacia gel) on a 1.25 by 26 cm column. The column was equilibrated with 0.025 mol/L of imidazole HCl buffer. Shrimp-water extract (2 gm in pH 4.0 polybuffer 74 HCl) was applied to the column and eluted with the polybuffer resulting in a pH gradient from 7 to 4. The pH gradient was followed by a linear sodium chloride gradient (0 to 4 mol/L in polybuffer). One milliliter column fractions were collected dropwise, analyzed for pH with a pH meter, for sodium chloride concentration with a conductivity meter, and for UV-absorbing material (280 nm) with a Beckman model 25 spectrophotometer (Beckman Instruments, Inc., Irvine, Calif.). Fractions were pooled according to absorption patterns of peaks and assayed for allergenic activity by testing undiluted, tenfold and 100-fold dilutions by RAST inhibition. Results were plotted on semilog paper, and INH-50 was calculated graphically. The total number of INH-50 units per sample were calculated by dividing total sample weight by its INH-50 unit.

RESULTS

Comparison of shrimp meat and water extracts

Five pounds of raw shrimp provided 760 gm of shelled, cooked shrimp meat. This meat yielded 1.46 gm of shrimp extract (determined by dry weight), whereas 12 gm of lyophilized material was obtained from the water in which the shrimp were boiled. Protein bands present in shrimp meat and water extracts were identified by IEF and Coomassie brilliant blue

staining. The results, illustrated in Fig. 1, demonstrate that extracts are remarkably similar, containing many proteins with acid pI. Shrimp-meat extract appears to contain additional protein bands with pIs at approximately pH 3.55 and 4.90, whereas bands at pI 4.0 are more intense in the water extract.

Allergenic activity of shrimp-meat and shrimp-water extracts was compared by determining the reactivity of 14 sera from skin test-positive subjects (Fig. 2). Although slight differences were noted in reactivity of several sera to the two shrimp extracts, linear regression analysis (r value, 0.959; $p < 0.0001$) demonstrated that the shrimp-specific serum IgE antibody reactivity of shrimp-sensitive individuals to both shrimp extracts was similar.

The allergenic activity of shrimp-meat and shrimp-water extracts was compared by RAST inhibition. Studies demonstrated that both shrimp-meat and shrimp-water extracts significantly inhibited shrimp meat and water RASTs (Fig. 3). The most inhibition was obtained in meat RAST with water extract. The least inhibition was of water RAST with meat extract. However, multiple linear regression analysis demonstrated no differences among the slopes and intercepts of the four RAST-inhibition curves.

Shrimp-specific IgE antibody reactivity of individual sera

Sera from 14 shrimp-sensitive, RAST- and skin test-positive individuals, as well as six atopic, shrimp-tolerant subjects who were shrimp skin test negative and RAST negative, were analyzed for IgE-antibody reactivity to shrimp-allergen components by immunoprinting. The shrimp RAST reactivity and to-

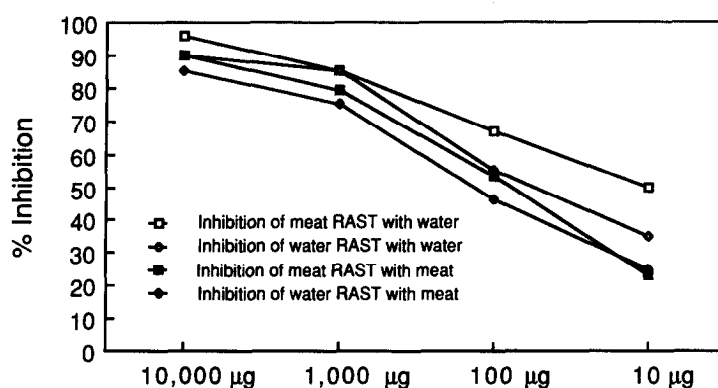


FIG. 3. Allergenic activity of shrimp-meat and shrimp-water extracts as compared by RAST inhibition. Increasing concentrations of shrimp meat or water extracts were tested for their ability to inhibit the shrimp meat or water RAST.

TABLE I. Shrimp RAST and total IgE levels of individual sera

Serum	RAST % label bound		Total IgE* IU/ml
	Shrimp water	Shrimp meat	
Skin test positive			
1	29.5	37.8	66
2	42.3	23.7	16
3	42.3	47.5	124
4	12.5	31.9	74
5	24.4	32.0	60
6	16.2	26.2	104
7	14.4	21.7	50
8	42.2	45.0	22
9	49.5	50.1	10
10	16.5	17.4	190
11	4.0	8.9	190
12	6.0	7.8	400
13	3.0	6.1	90
14	5.0	6.3	22
Skin test negative			
1	0.6	0.6	62
2	0.0	0.3	43
3	0.5	0.2	43
4	0.0	0.7	95
5	0.4	0.6	20
6	2.5	0.3	25

*These values correspond to the IgE concentration in diluted sera used in immunoprint determination.

tal IgE concentrations for these sera are summarized in Table I. The immunoprint results (Fig. 4; Table II) demonstrate that most sera from (12/14, 86%) shrimp-sensitive individuals contain detectable shrimp-specific antibodies, whereas no shrimp-specific antibodies were detected in sera from shrimp-tolerant subjects. In general, sera with higher RASTs yielded greater radiostaining.

When results were analyzed and tabulated in the allergogram (Table II), 86% of the skin test- and RAST-positive individuals reacted with shrimp-allergen proteins having pIs of 5.5 to 6.0. Reactivity for individual sera varied from three to seven bands within this region. Fifty percent or more of the patients' sera reacted with proteins that focused between pH 4.5 to 6.0 of the gel. Although the staining in-

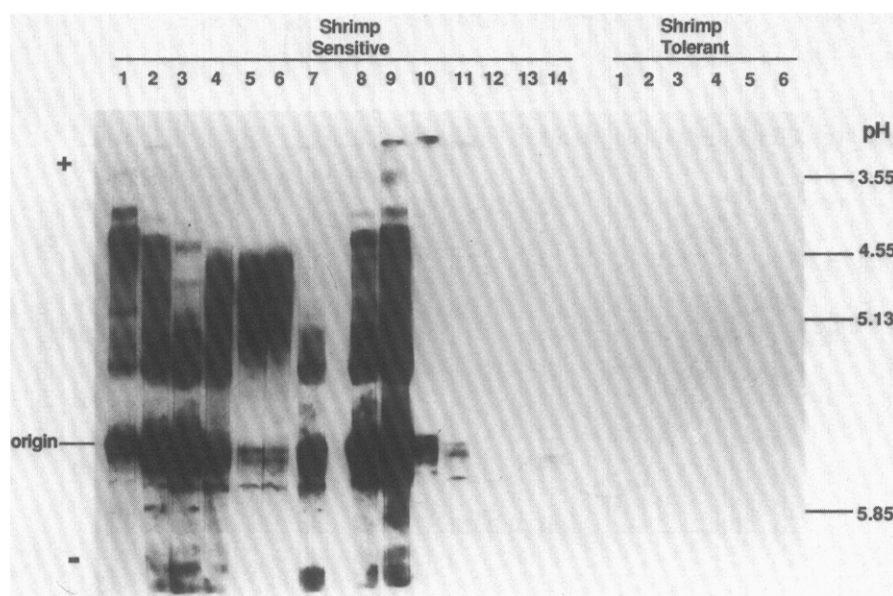


FIG. 4. IgE antibody reactivity of individual sera to shrimp allergens as detected by immunoprint. Fourteen RAST-positive sera (diluted 1:5 or 1:40) and six RAST-negative sera (diluted 1:2) were tested for IgE antibody reactivity to shrimp allergens after IEF.

TABLE II. Allergogram of shrimp-positive sera

Serum tested	<3.5	3.5-4.0	4.0-4.5	4.5-5.0	5.0-5.5	5.5-6.0
1	x	x	xxx	xx	xxxx	xxx
2		xx	xxxx	xxxx	xxxx	xxxxxx
3			xx	xx	xxxxxxx	xxxxxx
4				xxx	xxxxxx	xxxxxx
5				xxx	xxxx	xxx
6				xxx	xxx	xxx
7					x	xxxx
8				xx	xxxxxxxx	xxxxxx
9	x	x	xxxx	xxxx	xxxxxxxx	xxxxxxxx
10						xxxx
11						xxx
12						
13						
14						xxx
Total No. of reactors		2	4	8	9	12
% Reactivity		14.2%	28.5%	57.75%	64.3%	85.7%

tensity and number of bands detected by individual sera varied, most sera reacted with components in this pH range. Curiously, significant radiostaining occurred in the nitrocellulose membrane that corresponded to regions of the Coomassie-stained gel with little or no detectable protein bands.

Fractionation of shrimp extracts

The results of shrimp water-extract chromatofocusing are summarized in Fig. 5. The initial pH gra-

dient eluted one major and five minor peaks of UV-absorbing material with varying affinities for the gel. A somewhat surprising observation was the fact that a significant amount of material remained bound to the column. Three major peaks of UV-absorbent material were eluted with the sodium chloride gradient.

Column fractions comprising each peak were pooled and assayed for allergenic activity by RAST inhibition. Of the peaks eluted with the pH gradient, 12.5% and 10.3% of total allergenic activity recovered

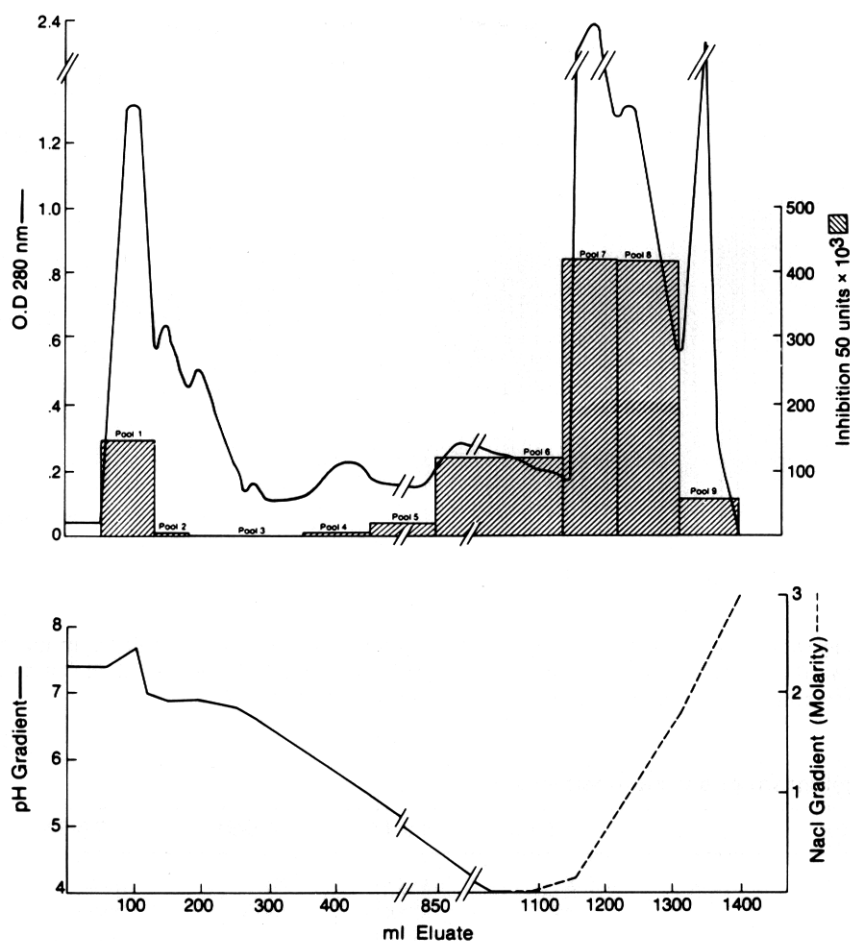


FIG. 5. Chromatofocusing of shrimp extract. Two grams of shrimp water in 25 ml 0.025 mol/L imidazole HCl, pH 7.4, were applied to a column (1.25 by 26 cm, 120 ml bed volume). The column was eluted with polybuffer 74 HCl, pH 4.0, until the effluent reached pH 4. After completion of the pH gradient, a linear NaCl gradient (0 to 4 mol/L) in polybuffer was applied. Column fractions were analyzed for protein by UV absorption, pH by pH meter, salt concentration by a conductivity meter, and allergenicity of pooled fractions by the RAST inhibition.

was present in pools 1 and 6, respectively; the remaining peaks contained only minor allergenic activity (<2% total activity recovered). Peaks 7 and 8, eluted with the NaCl gradient, contained significant RAST-inhibition activity and accounted for >70% of all the activity recovered from the column. Minor activity (4.5%) was present in pool 9.

Column pools 1 to 9 were analyzed for proteins by IEF and for IgE binding components by immunoprinting (Figs. 6 and 7). Although some shrimp proteins were present in peaks 1, and 3, clearly significant amounts of acidic proteins with pIs of 4.5 to 5 were present in fractions 5, 6, and 7. Many proteins present in pools 6 and 7 bound IgE antibodies from the pool of RAST-positive sera.

DISCUSSION

In most studies of crustacea allergy, allergen extracts are prepared from the edible portion of the boiled crustacean by homogenization of the meat in an appropriate extraction solvent, since this is the portion of the crustacea to which most sensitized individuals are exposed. However, more recently, there has been increasing evidence that the water in which crustacea are cooked may in fact contain significant amounts of allergens. Recently, Cartier et al.²⁰ have demonstrated significant allergenic activity present in the crab boiling fluid found in an occupational setting. A number of crab allergens have been identified in crab boil water by Bush et al.²¹ Our recent study demonstrated marked similarities of the antigens in

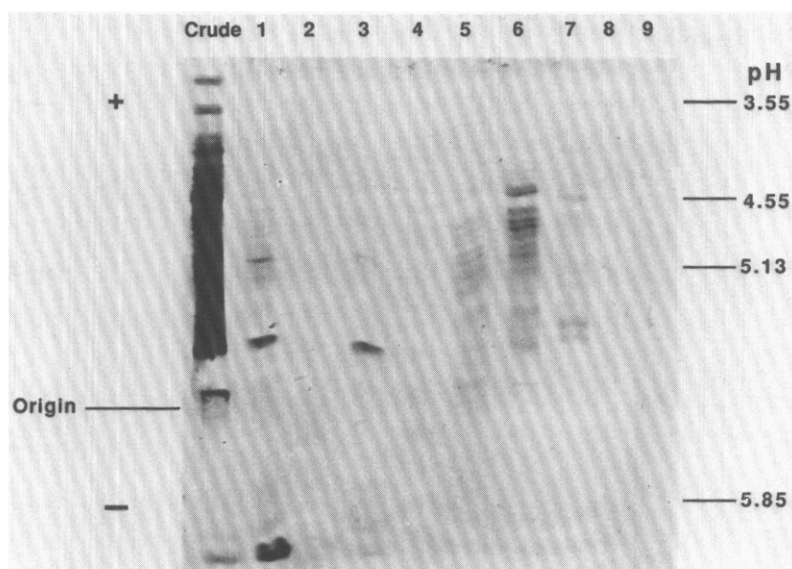


FIG. 6. Analytical IEF of shrimp after chromatofocusing. Unfractionated (*crude*) shrimp-water extract, and chromatofocused fractions (see Fig. 5) were electrofocused across the pH range of 4 to 6.0. On completion, the gel was fixed and stained with Coomassie brilliant blue.

crayfish (crawfish) boil fluid and crayfish meat.¹¹ For these reasons, we chose to analyze the shrimp boiling water for the presence of allergens and to compare the allergenicity of this fluid with the fluid of meat extracts.

Allergens present in shrimp water, although they are isolated by procedures different from procedures used for shrimp-meat allergens, appear to be similar to meat allergens. Based on IgE-antibody reactivity and RAST-inhibition studies, we conclude that the water in which shrimp are boiled is indeed a good source of large quantities of shrimp allergens. This fact has important implications for patient sensitization. Many subjects who complain of the odor of crustacea, while crustacea are being cooked, may actually be sensitized to aerosolized allergen released during the cooking process. Thus, highly susceptible shrimp-sensitive individuals may have to avoid areas where crustacea are boiled, in addition to abstaining from eating them.

Using these aqueous extracts of shrimp, we analyzed patient reactivity by the immunoprint procedure. IEF results demonstrated that shrimp proteins from either shrimp meat or boiling water appeared to migrate primarily in the acid pH range approximately from pH 4.0 to pH 6.5. Immunoprint analysis demonstrated that 12 of the 14 skin test-positive patients tested had significant IgE-antibody reactivity to shrimp-protein bands from boiling water. None of the shrimp allergens had alkaline pI (data not presented).

These reactions appear to be highly specific for shrimp allergens, since none of the control sera from skin test-negative and RAST-negative, shrimp-tolerant subjects demonstrated significant reactivity, although total IgE levels were quite similar. Immunoprint reactivity did not correlate with total serum IgE levels in the shrimp-sensitive group but did generally correlate with RAST reactivity.

Clearly, those shrimp allergens present in the pH range from 5.5 to 6.0 are of particular interest, since 86% of our patients' sera reacted with these proteins. Multiple bands appear to be present in these regions, although the number is difficult to assess because of the intensity of the reaction. It is quite possible that these bands might represent individual allergens or, more likely, isoallergens that migrate in a similar, but not identical, fashion. In contrast, very few weakly staining Coomassie brilliant blue bands were detected in this region, demonstrating differences in the sensitivity of these two methods.

Our results are similar to results of Hoffman et al.⁹ who identified a shrimp allergen (antigen II) with a pI of 4.75 to 5. Antigen II was reported to be a major shrimp allergen with a molecular weight of 38 kd. However, our studies differ in that we also detected significant reactivity to allergens with other pIs. The observation of multiple shrimp allergens are supported by crossed-radioimmunoelectrophoretic studies of shrimp allergens that demonstrated at least three major allergens reactive with >75% of sera tested.¹⁰ The

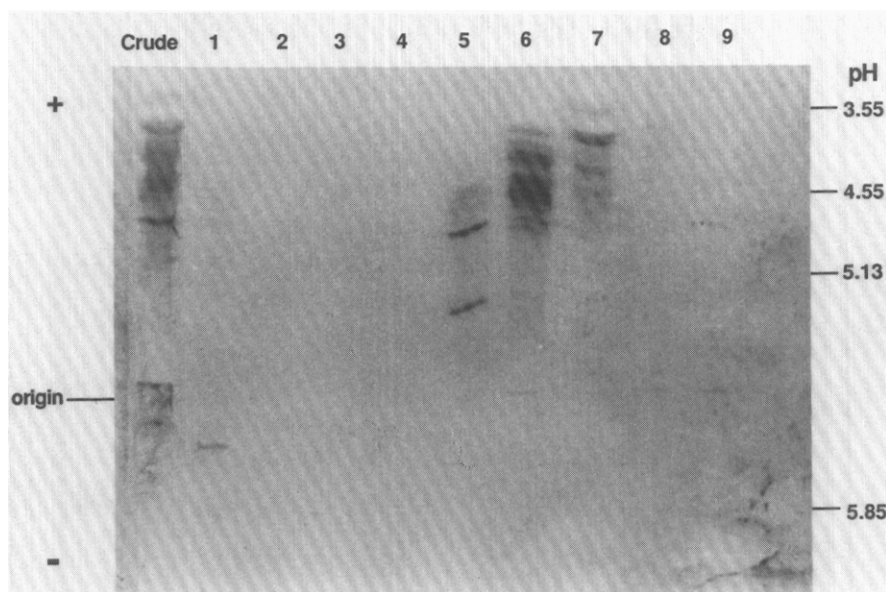


FIG. 7. Identification of IgE reactive shrimp-allergen bands by immunoprint method. IEF of shrimp fractions was performed as described in Fig. 6. The gel was blotted by passive transfer to nitrocellulose membrane, and IgE-reactive bands were identified by standard procedures.

discrepancy between our results and results of Hoffman et al.⁹ may be due to the fact that only one serum was used during the isolation process for antigen II. Although a number of patients appear to react with this allergen, we can postulate that other allergens may have been excluded during its purification because of the narrow reactivity of the test serum used.

The chemical nature of the shrimp allergens to which our patients reacted is of considerable interest. Recently, Nagpal et al.¹³ demonstrated a shrimp allergen that was resistant to protease digestion and yet sensitive to RNAase *t*2. Their study suggested that it was shrimp RNA and was thus the first report of a nucleic acid allergen. However, they were not able to prepare an RNA preparation completely devoid of protein, and thus, it is possible that their patients reacted to residual protein fragments bound to the RNA. Furthermore, since only two patients' sera were used, the clinical significance of this reactivity has yet to be determined. In our study, we have demonstrated that patients' sera react to bands that bind to nitrocellulose and which stain with Coomassie brilliant blue. Both activities are characteristic of proteins, although chemical analysis of these components must be performed to confirm that they are protein molecules.

To purify allergenic components in shrimp extracts, fractionation studies were performed. Gel filtration analysis (data not presented) confirmed observations by other investigators as to the heterogeneity of shrimp extract.⁹ Chromatofocusing of shrimp-water allergens

achieved significant purification. Most allergenic activity bound to the column, even at a pH of 4, which was below the pI for most allergens, as determined by immunoprinting. These allergenic components were eluted with a salt gradient suggesting that they are more hydrophobic, as compared to other components eluted earlier from the column. Clearly, there are discrepancies in the immunoprinting and chromatofocusing data with regard to pI of various shrimp fractions. This finding may be due to the fact that both the solid and liquid phases contribute to pH in chromatofocusing, and yet only the pH of the liquid eluate is measured. Chromatofocusing appears to be a promising procedure for further allergen fractionation in that significant amounts of allergenic activity were present in two peaks with strong affinity for the resin. However, IEF and immunoprinting demonstrated that these peaks contain a number of Coomassie brilliant blue staining protein bands, many of which bind IgE antibodies in patients' sera, and thus, further purification is necessary.

In summary, our results demonstrate that shrimp boiling fluid is an excellent source of shrimp allergens. Furthermore, immunoprinting provides a good technique for analyzing patient reactivity to these allergens as well as identifying them during purification. Shrimp allergens appear to migrate primarily in an acid pH range, and most shrimp allergens have pIs of approximately 4.5 to 5.8. This range includes the range reported for antigen II by Hoffman et al.⁹ However,

contrary to their findings, we also identified other shrimp allergens. This finding is in agreement with our earlier crossed radioimmuno-electrophoresis analysis that demonstrated a number of precipitin bands that bind IgE antibodies. Further studies will continue to characterize and analyze shrimp allergens. It is our aim to isolate purified shrimp allergens with which we can develop better diagnostic tests and treatment modalities for shrimp-sensitive patients.

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REFERENCES

1. Norman PS. Quality control of allergen extracts [Editorial]. *J ALLERGY CLIN IMMUNOL* 1982;69:1-2.
2. Norman PS. Why standardize extracts [Editorial]? *J ALLERGY CLIN IMMUNOL* 1986;77:405-6.
3. Goldstein GB, Heiner DH. Clinical and immunological perspectives in food sensitivity. *J ALLERGY CLIN IMMUNOL* 1970;46:270-91.
4. Aas K. The diagnosis of hypersensitivity to ingested foods. *Clin Allergy* 1978;8:39-50.
5. Aukrust L, Apold J, Elsayed S, et al. Crossed-immuno-electrophoretic and crossed-radioimmuno-electrophoretic studies employing a model allergen from codfish. *Intl Arch Allergy Appl Immunol* 1978;57:253-62.
6. Hoffman DR. The specificities of human IgE antibodies combining with cereal grains. *Immunochemistry* 1975;12:535-8.
7. Moroz LA, Yang WH. Kunitz soybean trypsin inhibitor. *N Engl J Med* 1980;302:1126-31.
8. Sachs MI, Jones RT, Yunginger JW. Isolation and partial characterization of a major peanut allergen. *J ALLERGY CLIN IMMUNOL* 1981;67:27-34.
9. Hoffman DR, Day ED, Miller JS. The major heat-stable allergen of shrimp. *Ann Allergy* 1981;47:17-22.
10. Lehrer SB. The complex nature of food antigens: studies of cross-reacting crustacea allergens. *Ann Allergy* 1986;57:267-72.
11. Halmeppuro L, Salvaggio J, Lehrer SB. Studies of allergens present in crawfish and lobsters. *Intl Arch Allergy Appl Immunol* 1987;84:165-72.
12. Lehrer SB, McCants MJ. Reactivity of IgE antibodies with crustacea and oyster allergens: evidence for common allergenic structures. *J ALLERGY CLIN IMMUNOL* 1987;80:133-9.
13. Nagpal S, Metcalf DD, Subba Rao PV. Identification of shrimp-derived allergen as *t* RNA. *J Immunol* 1987;138:4169-74.
14. Nagpal S, Rejappa L, Metcalf DD, Subba Rao PV. Isolation and characterization of heat-stable allergens from shrimp (*Penaeus indicus*). *J ALLERGY CLIN IMMUNOL* 1989;83:26-36.
15. May CD, Bock SA. Adverse reactions to food due to hypersensitivity. In Middleton E Jr, Reed CE, Ellis EF, eds. *Allergy: principles and practice*. St. Louis: CV Mosby, 1978:1159-71.
16. Cartier A, Malo J-L, Forrest F, et al. Occupational asthma in snow crab-processing workers. *J ALLERGY CLIN IMMUNOL* 1984;74:261-9.
17. Waring NP, deShazo RD, Daul CB, et al. Hypersensitivity reactions to ingested crustacea: clinical evaluation and diagnostic studies in shrimp-sensitive individuals. *J ALLERGY CLIN IMMUNOL* 1985;76:440-5.
18. Daul CB, Morgan JE, McCants ML, et al. Crustacea allergy: immunologic evaluation of shrimp-allergic individuals. *J ALLERGY CLIN IMMUNOL* 1987;80:716-22.
19. Ceska N, Lundkvist B. A new and simple radioimmunoassay method for determination of IgE. *Immunochemistry* 1972;9:1021-31.
20. Cartier A, Malo JL, Ghezzi H, et al. IgE sensitization in snowcrab-processing workers. *J ALLERGY CLIN IMMUNOL* 1986;78:344-8.
21. Bush RF, Meier-Davis S, Lehrer SB, et al. Snow crab asthma: identification of allergens by immunoblotting [submitted]