

Occupational IgE-mediated sensitization and asthma caused by clam and shrimp

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Background: To confirm occupational asthma caused by clam and shrimp in a food company worker, the following investigation was planned in 60 other exposed workers (56 participants).

Methods: Before the production period of clam and shrimp, a medical and occupational questionnaire was carried out and skin and RAST testing were done with common inhalants and clam, shrimp, crab, and lobster extracts. During the production period, environmental monitoring was performed with personal and general samplers; inhalation testing with methacholine was proposed to subjects with immediate skin reactivity to clam, shrimp, or both. After the production period, all subjects with an immediate skin reaction to clam, shrimp, or both and either a history of rhinoconjunctivitis, asthma, or bronchial hyperresponsiveness were seen by a specialist.

Results: Including the index case in whom occupational asthma to clam and shrimp had been confirmed, four (7%) subjects had a history of rhinoconjunctivitis and two (4%) had a history of asthma during the period of clam production, whereas three (5%) subjects had rhinoconjunctivitis and two (4%) had asthma during the shrimp production. Three (5%) subjects had immediate skin reactivity to clam, and nine (16%) subjects to shrimp. Four (7%) subjects had increased specific IgE antibodies (RAST binding $\geq 3\%$) to clam and eight (14%) to shrimp. Significant associations were found between immunologic reactivity to clam and shrimp on the one hand and to crab and lobster on the other. Environmental monitoring demonstrated clam and shrimp on the air sampling filters. Occupational asthma caused by clam was confirmed by specific inhalation challenges in one subject in addition to the index case. These two subjects had skin reactivity and increased specific IgE antibodies to clam, shrimp, or both.

Conclusion: By including the initial subject, the prevalence of immediate sensitization is 5% to 7% to clam and 14% to 16% to shrimp. Two (4%) subjects had occupational asthma caused by clam, and one (2%) had occupational asthma caused by shrimp. (*J ALLERGY CLIN IMMUNOL* 1995;96:608-17.)

Key words: Bronchial diseases, environmental monitoring, hypersensitivity, occupational diseases, RAST

Seafood and fish are common causes of food allergies.^{1,2} They are also responsible for occupa-

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Abbreviations used

FEV₁: Forced expiratory volume in 1 second
PC₂₀: Provocative concentration of methacholine causing a 20% fall in FEV₁

tional asthma in processors of food.³ An oyster contaminant, a protochordae called "hoya" or "sea-squirt,"⁴ prawn,⁵ and crab⁶ are causes of occupational asthma in the seafood industry. To our knowledge, only one case of occupational asthma caused by proteins derived from shrimp has been described,⁷ whereas clam has not been reported as a cause of occupational asthma.

We recently confirmed the diagnosis of occupational asthma caused by clam and shrimp in a food processing worker. The aim of this study was to assess the prevalence of occupational IgE-mediated sensitization and asthma in the other processors of food who handled lyophilized clam and shrimp within the same facilities. The levels of clam and shrimp antigens present in the working environment were also assessed.

METHODS

Subjects

After the diagnosis of occupational asthma caused by clam and shrimp was confirmed in one subject with specific inhalation challenges (see following), we recruited all other workers ($n = 60$), who were exposed to clam and shrimp proteins in a food company that processes these agents in lyophilized form, as participants in this study. Only four subjects could not be reached, because they were on pregnancy leave. All subjects were exposed intermittently to clam and shrimp, which was processed in batches 1 or 2 days at a time. Total durations of production period to either clam, shrimp, or both were 189 hours in 1992 and 294 hours in 1993; the proportions of clam and shrimp productions were equivalent. During the production periods lyophilized powder extracts of various foods were put into bags. Foods in powder form were first prepared in conic reservoirs equipped with blenders. The resulting food powder was then put into bags before being stored. Twenty-five employees worked on the bag production line.

Study design

Survey, part I. A questionnaire was administered by a technician, and skin testing was done by a nurse on all 57 subjects. Sera from 55 subjects were available for immunologic testing.

Survey, part II. Spirometry and assessment of bronchial responsiveness was planned in all subjects who showed skin reactivity to clam, shrimp, or both.

Survey, part III. A medical assessment was carried out in all subjects with skin reactivity to clam, shrimp, or both and (1) either a history of rhinoconjunctivitis or asthma during the production period, or (2) bronchial hyperresponsiveness to methacholine. Specific inhalation challenges were then considered.

Questionnaire

A standardized asthma questionnaire⁸ with added questions on occupational items that had been used in previous studies⁹ was administered by a trained technician to every worker. At this step asthma was diagnosed in the presence of two or more positive answers on wheezing, chest tightness, dyspnea, cough either spontaneously or on exercise and after inhaling nonspecific irritants. Rhinitis was defined by the presence of at least

one symptom (runny nose, congestion, sneezing). Conjunctivitis was defined by the presence of at least one symptom (itchy eyes, redness of the eyes, eye watering). Occupational asthma was defined from the questionnaire by the presence of at least one symptom (dyspnea, cough, wheezing) during a specific production period.

Skin testing

Skin tests were done with seven common inhalants (tree, grass and ragweed pollens, house dust, *Derмато-phagoides farinae*, cat and dog danders) (Omega, Montreal, Canada). Control diluent and histamine phosphate (1 mg/ml) tests were performed with the prick method. Commercial preparations (Omega) of clam, shrimp, lobster, and crab were also used. Finally, extracts of clam (1 and 10 mg/ml), shrimp (1 and 10 mg/ml), and guar gum (1 mg/ml) were prepared in phosphate-buffered saline solution and used for skin prick test. A positive reaction was defined by the presence of a wheal of 3 mm or greater with a wheal reaction to histamine phosphate of 3 mm or greater and a negative (<3 mm) reaction to the diluent.

Specific IgE assessments

RASTs were used as previously described¹⁰ to quantify circulating IgE in the test sera of 55 of the 57 workers. Serum samples were coded, and the workers responsible for the tests (M. M. and S. L.) were not aware of the clinical findings at the time of analysis. Clam and shrimp provided by the employer, and shrimp, lobster, crawfish, and crab phosphate-buffered saline solution extracts were coupled to CNBr-activated filter paper disks (1 mg/disk) prepared by the method of Ceska and Lundkvist.¹¹ Guar gum was coupled at 0.5 mg/disk. One hundred microliters of ¹²⁵I-labeled anti-IgE containing approximately 15,000 cpm (Pharmacia, Piscataway, N.J.) was added to the tubes. RASTs were performed in duplicate, and results were expressed as mean percent binding. RAST ratios greater than 3% binding were considered to be positive. Specific IgE measurements to clam and shrimp were performed for all 55 workers. Those sera with positive RAST to clam, shrimp, or both only ($n = 9$), and five negative control sera (from exposed workers with negative RAST results to clam, shrimp, or both) were further tested for IgE antibodies to lobster, crawfish, and crab extracts.

Spirometry and assessment of bronchial responsiveness to methacholine

Spirometry was assessed according to the criteria of the American Thoracic Society,¹² and bronchial responsiveness was assessed by nebulizing methacholine at tidal volume breathing for 2 minutes with a Wright nebulizer (Ferraris Medical, Inc., Holland, N.Y.) (output = 0.14 ml/min).¹³ The provocative concentration causing a 20% fall forced expiratory volume in 1 second (FEV₁) (PC₂₀) was obtained. Bronchial hyperresponsiveness was considered significant at a PC₂₀ level of 16 mg/ml or less.¹⁴

TABLE I. Anthropometric, clinical, and functional results

Sex (male/female)	37/20
Age (mean $\pm$ SD yr)	31 $\pm$ 7
Duration of employment (mean $\pm$ SD yr)	4.8 $\pm$ 3.4
Smoking habits (smokers/ex-smokers/nonsmokers)	29/20/8
At least two symptoms suggesting personal asthma (%)	15 (26)
Symptoms on exposure to clam	
Rhinoconjunctivitis (%)	4 (7)
Asthma (%)	2 (4)
Symptoms on exposure to shrimp	
Rhinoconjunctivitis (%)	3 (5)
Asthma (%)	2 (4)
Atopy*	21
Immediate skin reactivity to	
Clam (%)	4 (7)
Shrimp (%)	9 (16)
Increased specific IgE antibodies to†	
Clam (%)	4 (7)
Shrimp (%)	8 (14)
Occupational asthma	
Caused by clam‡	2
Caused by shrimp	1

*At least one immediate skin reaction to 15 common inhalants by the prick method.

†RAST ratios >3% binding.

‡One subject positive to both clam and shrimp.

Environmental monitoring

Environmental monitoring was carried out with personal samplers at six different workplaces and fixed samplers at two stationary samplers in the working area. The air sampling was collected on personal filters with pumps running at a constant flow of 1.5 L/min for periods varying from 113 to 238 minutes depending on the sample. Stationary samplers were run at a flow of 50 L/min. Filters were analyzed in two ways: (1) under microscopy with polarized light in which particles have a distinct aspect that allows for their identification and (2) with RAST inhibition. For RAST inhibition filters were cut into 3 $\times$ 3 mm pieces, vortexed for 1 minute in 1.5 ml 0.1 mol/L phosphate-buffered saline solution (pH = 7.2), and mixed on a magnetic stirrer overnight at 4° C. Samples were centrifuged (8000 g) for 1 minute. Approximately 1 ml of supernatant fluid was recovered from each sample. Samples were assayed for protein content with the Pierce Micro Protein assay (1 to 25 μ g range). For RAST inhibition lyophilized shrimp and clam material were first suspended in 0.1 mol/L borate buffer (pH = 8.0) and mixed overnight at 4° C. Guar gum was extracted with borate buffer for 4 hours at room temperature, clarified through cheesecloth, and concentrated with Ficoll (Pharmacia, Uppsala, Sweden).

All samples were centrifuged (90,000 g for 30 minutes at 4° C), and the supernatant fluid was saved. A serum pool of three subjects who had increased specific IgE levels to shrimp, clam, and guar gum was diluted, and assays were done by RAST to determine the optimal dilution of RAST inhibition assays. To assess inhibition assay specificity, shrimp and clam extracts were coupled to CNBr-activated disks (1.0 mg/disk, dry weight). Shrimp and clam disks were inhibited with shrimp, clam, and guar gum at tenfold dilutions (100 to 0.001 μ g dry weight). Fifty microliters of inhibitor and 50 μ l of serum pool (1:10 for shrimp and 1:40 for clam) were added to the shrimp or clam disks and incubated overnight on a rotator at room temperature. ¹²⁵I-labeled equine anti-human IgE (15,000 cpm/disk) were added to disks washed with saline solution and incubated on a rotator overnight at room temperature.

Specific inhalation challenges

Exposure to clam and shrimp dry powder was carried out with a closed-circuit particle generator, which allows for steady and nonirritant concentrations of dusts.¹⁵ In one case clam powder was diluted in phosphate-buffered saline solution and aerosolized with a Wright nebulizer (output = 0.14 ml/min) at tidal volume breathing. Dilutions of 1:64, 1:16, and 1:4 were used.¹⁶

Statistical analysis

Fisher's exact probability test was used to evaluate associations, and a *p* value of less than 0.05 was considered significant.

RESULTS

Survey, Part I

Questionnaire. Including the initial subject, 57 workers (36 men and 21 women) participated in the study (Table I). There were 25 operators working on the production line and 12 technicians responsible for quality control. Only nine subjects were older than 40 years of age. Eighteen subjects had worked for 3 to 5 years, and 19 had worked from 5 to 10 years. Fifteen (26%) subjects had at least two symptoms that suggested personal asthma (spontaneous wheezing, chest tightness, cough or dyspnea, at least two of the preceding symptoms on exercise or on exposure to heavy smell, dust, and smoke). Eight subjects reported having been told by their physician that they had asthma, and six of these had symptoms suggesting personal asthma (see above). Less than 10% of workers reported either rhinoconjunctivitis, asthma symptoms, or both on specific exposure to clam or shrimp (Table I).

Skin testing and specific IgE assessments. Four subjects, including the initial subject, had immediate

TABLE II. Clinical, functional, and immunologic results of subjects with skin reactivity, increased specific IgE antibodies, or both to clam, shrimp, or both

Subject No.	Skin reactivity		Atopy	History of asthma	History of occupational		PC ₂₀ (mg/ml)	Specific IgE		Specific inhalation challenges
	Clam	Shrimp			Asthma	Rhinoconjunctivitis		Clam	Shrimp	
Skin reactivity to clam, shrimp, or both with increased specific IgE antibodies										
14	--	+	-	-	-	+	>32	Normal	Increased	ND
15	-	+	+	-	-	-	ND	Normal	Increased	ND
17	-	+	+	+	-	-	12 (6)	Normal	Increased	Negative
21	-	+(-)	+	+	-	+	14	Increased	Increased	ND
39	+	+	+	-	+	+	>32	Increased	Increased	+(Clam)
57	+	+	+	-	+	+	0.3	Increased	Increased	+(Clam and shrimp)
Skin reactivity to clam, shrimp, or both with normal specific IgE antibodies										
11	-	+	+	-	-	-	ND	Normal	Normal	ND
16	-	+	+	-	-	-	>32	Normal	Normal	ND
22	+(-)	-	+	+	-	-	0.4	Normal	Normal	ND
41	+	+	+	+	-	-	>32	Normal	Normal	ND
Increased specific IgE antibodies to clam, shrimp, or both without skin reactivity										
5	-	-	+	+	-	+	ND	Increased	Increased	ND
40	-	-	+	-	-	-	ND	Normal	Increased	ND

Subject no. 14 had a history of general anaphylaxis reaction when ingesting a variety of crustacea; specific inhalation challenges were therefore not performed. Subject no. 15 refused methacholine inhalation testing. Information in parentheses refers to tests that were repeated at the time of referral to a specialist (subjects no. 21 and 22) and specific inhalation challenges (subject no. 17). ND, not done.

skin reactivity to clam (Table II). Nine subjects reacted to the shrimp extract. No subject reacted to guar gum. Five subjects had skin reactivity to lobster, and five to crab. Three of the four subjects who reacted to clam also reacted to shrimp. Five of the nine who reacted to shrimp also reacted to the commercial extract of lobster, and four of them reacted to the commercial extract of crab. All subjects who reacted to clam were atopic, and eight of the nine subjects who reacted to shrimp were also atopic (Fisher's exact probability tests for association between atopy and skin reactivity to clam, shrimp, crab, and lobster all significant).

Four subjects had significantly increased specific IgE levels to clam (Table II). Three (75%) of these also had skin reactivity. Eight subjects had increased specific IgE levels to one or another of the two extracts of shrimp. Five (63%) of these also had positive skin test results. The four subjects with increased specific IgE levels to clam also had high levels of IgE antibodies to shrimp. Of the eight sera that had increased specific IgE levels to clam, shrimp, or both, five had increased levels to lobster, seven to crawfish, and six to crab. In five negative control sera with normal specific IgE levels to clam and shrimp, only one serum had significantly increased specific IgE level to crawfish

only, but not to lobster and crab. (Fisher's exact probability tests for association between increased specific IgE levels to clam, shrimp, or both on the one hand and increased specific IgE levels to crawfish, lobster, and crab on the other hand were all significant.) Three subjects had increased specific IgE levels to guar gum.

A serum pool from three subjects who had increased specific IgE levels to shrimp, clam, and guar gum was diluted, and assays were done by RAST to determine the optimal dilution for RAST inhibition assays. Two RAST assays were performed. Initially, the serum pool was tested undiluted, 1:10 and 1:100. Because the tenfold dilutions used in the first assay did not clearly establish the optimal serum pool dilution, the assay was repeated with intermediate dilutions. From RAST results the optimal serum concentration for clam RAST was determined to be 1:40 (38.8% binding); shrimp RAST, 1:10 (26.6% binding); and guar gum RAST, undiluted (8.4% binding).

The RAST inhibition assay was then performed to assess inhibition assay specificity. Inhibition results are summarized in Figs. 1 and 2, and the 50% inhibition doses are determined. Shrimp was inhibited 50% by 3.5 μ g of shrimp, 0.39 μ g of clam, and 2.0 of μ g guar gum. Clam was inhibited 50%

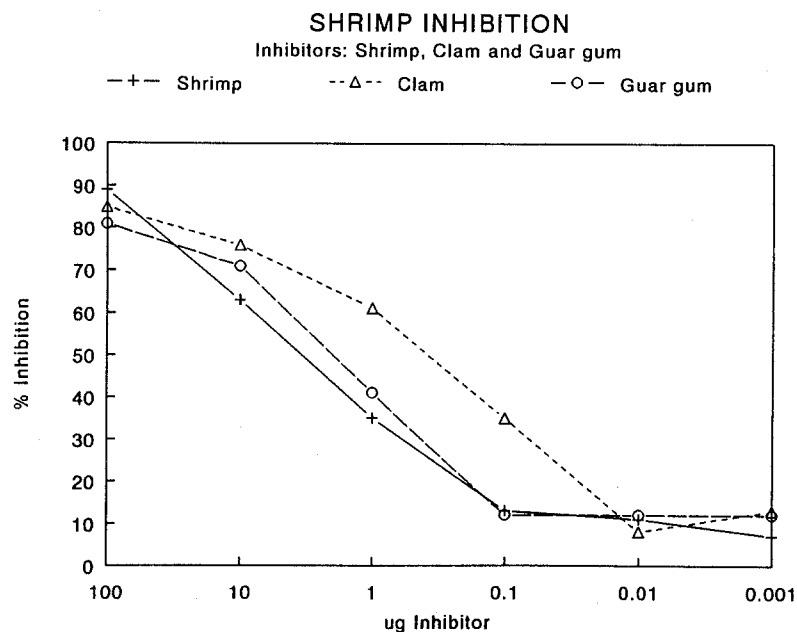


FIG. 1. Inhibition of shrimp RAST. Increasing concentrations of shrimp, clam, and guar gum extracts were tested for their ability to inhibit shrimp RAST. Maximal inhibition of 80% to 90% range was achieved with 100 μ g of inhibitor.

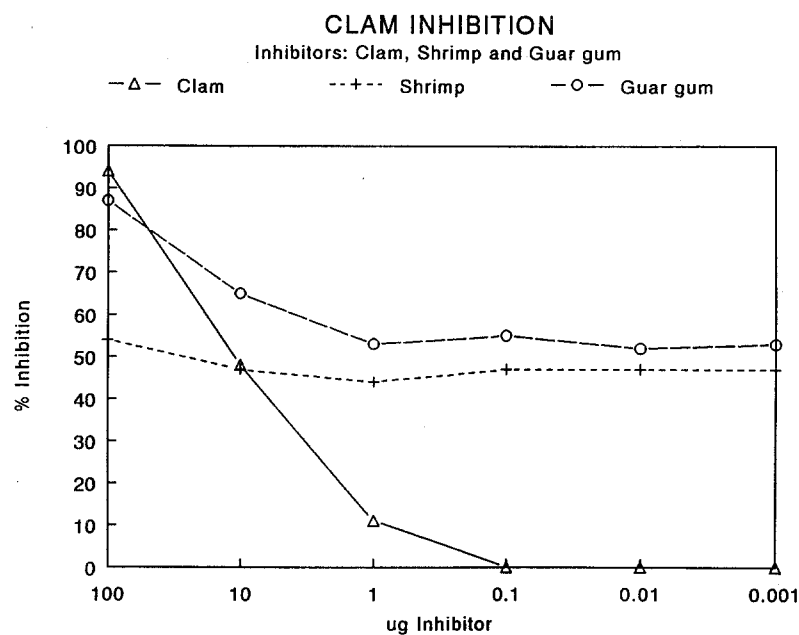


FIG. 2. Inhibition of clam RAST. Increasing concentrations of clam, shrimp, and guar gum extracts were used to inhibit clam RAST. Only clam extract yields significant dose-dependent inhibition. Maximal inhibition was achieved with 100 μ g with inhibitor.

by 11 μ g of clam and 29 μ g shrimp, whereas guar gum inhibition was not dose-dependent. The inhibitory effect of guar gum on shrimp and clam was thought to be nonspecific, because it was not related to shrimp or clam and did not always yield

a dose-dependent inhibition. An inhibition assay was therefore developed to test for nonspecific inhibition by guar gum. Soybean disks (1.0 mg/disk, dry weight) were incubated with 50 μ l of guar gum or soybean and 50 μ l of undiluted serum from a

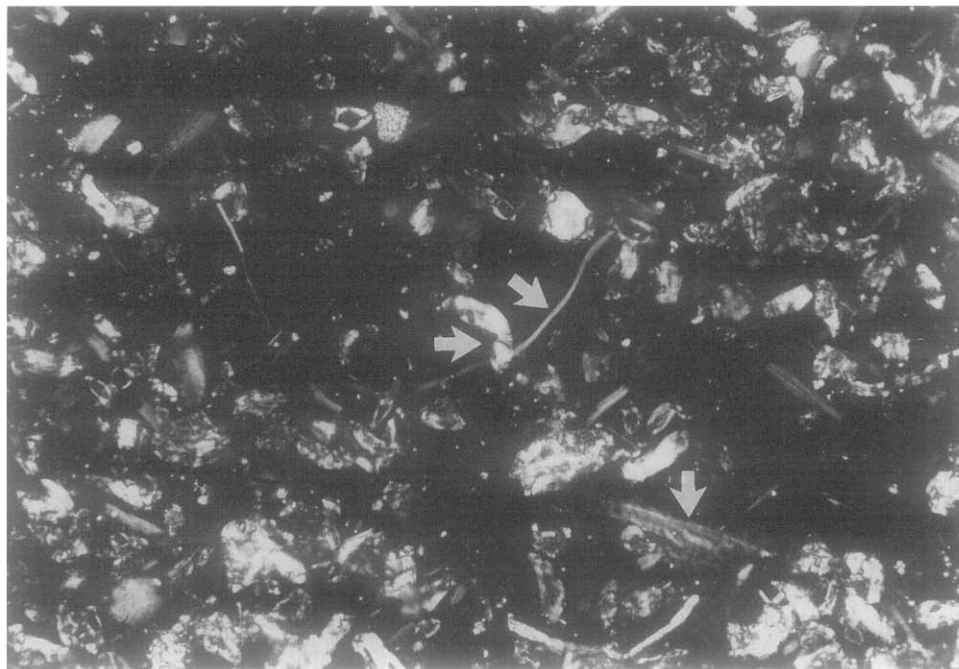


FIG. 3. Examination of environmental samples under polarized light. *Horizontal arrow*, guar gum dust; *vertical arrow*, clam dust; *diagonal arrow*, shrimp dust. Most of the dust sample was made of guar gum.

soy-sensitive, RAST-positive individual. Inhibitors were tested at tenfold concentrations (100 μ g to 0.001 μ g, dry weight). Significant inhibition (<50%) of soybean RAST was observed with guar gum. It is therefore concluded that this inhibition was nonspecific (not immunologic) and that the inhibition of shrimp and clam RAST by guar gum was also nonspecific.

Environmental monitoring. Examination of filters with microscopy under polarized light showed the presence of corn starch (95%), guar gum, cellulose, clam (traces), and shrimp (1%) dust in three of five filters; the other two filters contained an insufficient amount of dust for analysis (Fig. 3).

Fifty microliters of each undiluted filter extract was used in duplicate to inhibit clam and shrimp RAST disks. Results are summarized in Table III. The two most potent samples were obtained from the shrimp production area. These results suggest that allergens from the food production line are present in the atmosphere. The reason that inhibition to shrimp was greater in the clam production period on filters 1 and 2 might be related to the fact that although sampling took place in the afternoon of a clam production, shrimp had been handled in the morning in the same room.

TABLE III. Production filter inhibition of clam and shrimp

	Percent inhibition	
	Shrimp	Clam
Clam production		
Filter no. 1	41	12
Filter no. 2	19	16
Filter no. 3	4	7
Control filter	0	0
Shrimp production		
Filter no. 1	9	2
Filter no. 2	0	6
Control filter	0	7

Survey, part II

Seven of nine eligible subjects (subjects with skin reactivity to either clam or shrimp, excluding the initial subject) underwent methacholine inhalation testing 1 day after the onset of the clam production period (Table II). Three subjects had bronchial hyperresponsiveness ($PC_{20} \leq 16$ mg/ml). Because it was impossible to repeat the testing at the time of the shrimp production period, it was decided to refer to a specialist all subjects with skin reactivity to clam or shrimp and either (1) a history of rhinoconjunctivitis or asthma during the clam or

shrimp production period (two subjects) or (2) bronchial hyperresponsiveness (three subjects).

Survey, part III

Medical assessment and specific inhalation challenges. Besides the initial subject, five subjects were assessed by a specialist (J. L. M., A. C. or A. D.). Two subjects (Table II) had a history of asthma before starting to work for the food company. Skin testing with clam and shrimp were repeated, and results were judged as negative in these two subjects (these tests had produced a 3 mm wheal at the time of the initial survey at work). One subject (Table II) had a history of systemic allergic reaction after ingesting a variety of crustacea including shrimp, before starting to work. Because of the potential risk we decided not to do specific inhalation challenges. The one subject (Table II) with skin reactivity to shrimp only and a PC₂₀ methacholine value of 6 did not show significant reduction in FEV₁ after being exposed to shrimp dust for 2 hours in a progressive manner. The other subject (Table II) with skin reactivity to both clam and shrimp and a PC₂₀ methacholine value of 64 at the time of testing (1 month after the end of the production period) had a significant reaction to clam only. Results of specific inhalation challenges in this subject and in the subject who first presented are shown in Fig. 4.

DISCUSSION

This study shows that clam and shrimp are causes of IgE-dependent sensitization and occupational asthma in workers processing these agents. So far, although shrimp is a major seafood allergen in consumers, only one case of occupational asthma to shrimp meal has been described.⁷ We demonstrated that the reported subject, an aquaculture industry worker, had a dual asthmatic reaction with a maximum fall of 50% in FEV₁. Increased specific IgE antibodies were demonstrated, and the antigens seemed to be present both in the flesh and in the exoskeleton of shrimp. The frequency of sensitization and symptoms to shrimp was not established.

In this study the prevalence of IgE-mediated sensitization was 5% to 7% to clam and 14% to 16% to shrimp, depending on whether immediate skin reactivity or levels of specific IgE were used. These prevalence rates are lower than those reported in other cross-sectional studies of workers exposed to seafood derivatives. Gaddie et al.⁵ identified 13 of 50 (26%) subjects who demonstrated immediate skin reactivity and 8 of 50 (16%)

who had increased specific IgE levels to prawn (Norway lobster) in a producing factory. The frequency of immediate skin reactivity to crab was found to be 65 of 298 (22%) in two factories.⁶ Reasons for a lower prevalence of sensitization in our study can be related to one or another of the following factors: dry aerosol as opposed to wet aerosols in previous studies^{5,6} and different degrees of exposure, which is a function of the concentration and duration of employment. Although the concentration of airborne antigens was not established in previous studies, it was likely to be low in this investigation as determined by examining filters by microscopy under polarized light (<1% of the total concentration of dust was made of shrimp or clam) or through RAST inhibition, which yielded low values of antigens. The workers in this study had also been exposed intermittently to clam and shrimp. Although clam and shrimp were handled with equal frequency, the prevalence of sensitization was lower with clam. There are hypothetical explanations for this: first, shrimp can be aerosolized more readily than clam as shown by a percentage RAST inhibition more elevated for shrimp than for clam; second, the "sensitizing" properties of shrimp can be higher than for clam. Shrimp is regarded as one of the most potent of the seafood allergens in consumers.

Atopy was associated with IgE-mediated immunologic reactivity to clam and shrimp and to crab and lobster. The association has also been observed with other high-molecular-weight agents causing occupational asthma.¹⁷ We showed evidence of immunologic cross-reactivity between the various seafoods used in our study, in regard to both skin reactivity and specific IgE levels. Immunologic cross-reactivity between different crustaceans such as shrimp, crab, and lobster has been well documented.¹⁸ In addition, cross-reactivity between crustacea (shrimp, crab, lobster, and crawfish) and mollusks (oysters) has been reported, although the clinical significance is not clear.¹⁸ Recently, the major allergen in shrimp has been identified as shrimp tropomyosin.¹⁹⁻²¹ It would be of interest to identify those allergens to which shrimp workers with occupational allergies react. In fact, it is of considerable interest to compare the reactivity of individuals exposed to aeroallergens as compared with those of ingested allergens. Studying the reactivity of sera of these shrimp-sensitive workers in comparison with that of sera from shrimp-sensitive consumers should provide useful information.

The strategy we used to diagnose occupational

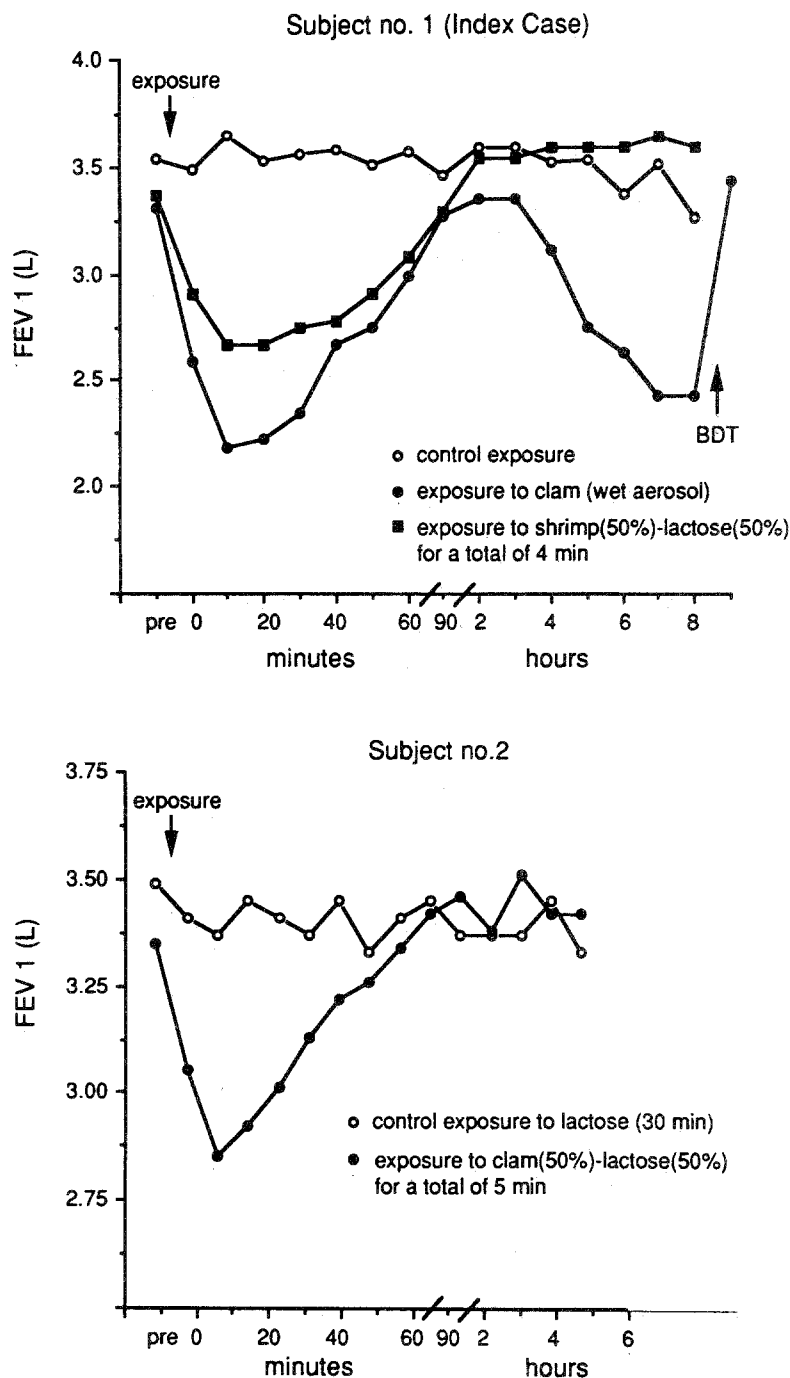


FIG. 4. Results of specific inhalation challenges in two subjects with occupational asthma, one to clam and shrimp (**upper panel**) and other to clam only (**lower panel**). BDT, Inhaled β -2 adrenergic agent. Durations of exposure are shown.

asthma was based on a progressively more stringent identification of cases as suggested by Bernstein.²² The original assumption was that this condition was IgE-mediated, because we dealt with a high-molecular-weight, protein-derived agent and skin reactivity was a reflection of increased specific IgE antibodies. Therefore subjects with occupa-

tional asthma caused by clam, shrimp, or both should demonstrate immediate immunologic reactivity to these agents. Immediate skin reactivity was used in our strategic plan as a reflection of IgE-mediated immunologic reactivity. Specific IgE levels were assessed at a later stage. Although a satisfactory correspondence was seen between skin

reactivity and specific IgE levels to clam and shrimp, three different subjects (Table II) had increased specific IgE levels to either clam or shrimp without demonstrating skin reactivity. Two of the three had symptoms of rhinoconjunctivitis during the relevant production period of clam, shrimp, or both, but neither had symptoms suggesting asthma. It is therefore not excluded that these subjects had occupational rhinoconjunctivitis caused by clam, shrimp, or both. The next step in our strategic screening was to identify the state of bronchial hyperresponsiveness at the time of a production period; the rationale was that having both skin test reactivity and bronchial hyperresponsiveness results in a very high likelihood of having occupational asthma, as shown to be 80% for psyllium, another high-molecular-weight agent.²³ Assessing bronchial responsiveness at the time of a production period proved awkward for two reasons: (1) subjects were exposed for only 1 or 2 days at a time, and this short exposure might not be sufficient to induce asthma or significant bronchial hyperresponsiveness; (2) it was difficult to plan the assessment of bronchial responsiveness during the clam and shrimp production period. We could only assess bronchial responsiveness after 1 day of the clam production period in seven of the 10 subjects with skin reactivity to clam, shrimp, or both. We therefore decided to also rely on the symptoms obtained from the initial questionnaire. All subjects with symptoms of rhinoconjunctivitis, asthma, or both were referred to a specialist. It is not excluded that some subjects had denied their symptoms and were overlooked. However, employees were told the general strategy of the investigation when the study started, and the impression was that there was little fear of losing a job, which would have resulted in denying symptoms.

The prevalence of occupational asthma caused by clam or shrimp found in this study was less than 5%. Except for a prevalence of occupational asthma of 16% that was found in a cross-sectional study, which included 97% of all workers ever exposed to crab,⁶ our previous cross-sectional surveys of employees exposed to high-molecular-weight agents showed that the prevalence rates were less than 5% with a case identification strategy that was close to the one used in this study.

In our study the environmental monitoring was carried out with personal samples in two ways: under microscopy with optical density and by RAST inhibition assay. Both showed the presence of clam and shrimp in the air at the time of the production

period, which supports the likelihood that this was the cause of sensitization and asthma. The quantity of antigen was small, however. Additional and longer air sampling might allow us to collect more antigen and could lead to the identification of the responsible proteins.

We conclude that exposure to clam and shrimp represents a significant risk in workers processing seafood. The extent of this risk must be examined in larger samples to explore the efficacy of control measures of exposure.

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