Failure of enzyme encapsulation to prevent sensitization of workers in the dry bleach industry

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BDE added to dry bleach have been associated with immunologic sensitization and development of clinical allergic disease in detergent workers and occasionally in consumers. However, improved dust control and modification of the manufacturing process through encapsulation of enzyme were believed to have reduced or eliminated these problems. To determine whether or not immunologic sensitization could still develop in the detergent industry, we studied employees of a dry bleach manufacturing plant that incorporated encapsulated BDE into a consumer product. We performed air sampling for enzyme dust and total particulates, administered questionnaires, conducted physical examinations, and spirometry in 13 currently exposed, two previously exposed and nine nonexposed, employees. To assess sensitization status, RAST and ELISA were performed. Air concentrations of enzyme dust ranged from 0.002 to 1.57 μg/m³; all of these levels were below the TLV of 3.9 μg/m³. Positive BDE-specific RAST results (3.4%, 4.4%, and 8.0% binding) were obtained in three of 12 currently exposed workers. Results of personal breathing-zone air sampling indicated that these workers had high dust-exposure levels. Specificity of RAST was verified by RAST inhibition with BDE. BDE-RAST binding was not significantly elevated in the nonworkers (range: 0.6% to 1.4% binding). Positive results for specific IgG by ELISA were obtained in four of 12 currently exposed and in one of two previously exposed workers but in none of the nonexposed workers. We conclude that immunologic sensitization can develop after occupational exposure to encapsulated BDE in the dry bleach industry. We have not proved, however, that this immunologic reactivity is related to clinical sensitivity. (J ALLERGY CLIN IMMUNOL 73:348-355, 1984.)

Proteolytic exoenzymes that are derived from strains of the *Bacillus* genus (e.g., *B. subtilis*) have been added to synthetic detergents to enhance cleaning effectiveness. These enzyme products were first produced in Holland in 1963 and were added to the formulation of soap products in the United States and the United Kingdom in 1967. In the late 1960s, 70% to 90% of all laundry detergents sold in the United States contained proteolytic enzymes.[1]

Allergic sensitization to bacterial exoenzymes came to be recognized as a common occurrence in detergent workers[1-7] and was described also in some consumers.[7-9] The spectrum of sensitization varied from the presence of positive-prick skin tests without clinical symptoms to a full range of respiratory symptoms that included nasal stuffiness, rhinorrhea, lacrimation, throat irritation, chest tightness, cough, and breathlessness.[1, 5, 6] Results of skin tests, bronchial provocation testing,[6] and studies for specific-IgE antibodies[7] confirmed the immunopathogenesis of these symptoms. Generally, the incidence of sensitization tended to be greater with exposure to higher levels of enzyme dust.

Recognition and elucidation of the problem of sensitization resulted by the early 1970s in the initiation of remedial actions including: (1) plant engineering measures to achieve stricter dust control and better exhaust ventilation, and (2) modification of manufac-

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Abbreviations used

BDE:	Bacillus-derived exoenzymes
cfm:	Cubic feet per minute
TLV:	Threshold limit value
ACGIH:	American Conference of Government Industrial Hygienists
RAST:	Radioallergosorbent test
ELISA:	Enzyme linked immunosorbent assay
PSF:	Phenylmethylsulfonyl fluoride
FEV_1:	Forced expiratory volume in 1 sec
PBS:	Phosphate-buffered saline
OD:	Optical density
BSA:	Bovine serum albumin

turing processes to develop coated or encapsulated (presumably nonrespirable) enzyme particles. One of the most significant improvements in the latter category was the invention of an enzyme granulate or marume. Marumes are uniformly sized solid spheres (approximately 600 μm in diameter) that contain enzyme that is embedded in a matrix of inorganic salts. The technique of marumerization was purported to reduce exposure to airborne-respirable enzyme dusts in the plant environment and thus to reduce the incidence of respiratory sensitization.

Most investigators reported a decline in the percentage of detergent workers becoming skin test positive as the recommended engineering improvements were incorporated. Weill et al.[1, 5] noted overall clinical improvement after a reduction of atmospheric enzyme concentrations. Juniper et al.[10] reported a steady diminution of acute respiratory illnesses by the end of 1971. At a 1976 symposium on the biological effects of proteolytic enzyme detergents, it was noted that no person who had started work after 1970 in one United States factory had become clinically sensitized.[11]

The present investigation reports the results of a cross-sectional clinical and immunologic study that was conducted at a dry bleach manufacturing company that used encapsulated (marumerized) bacterial enzymes.[12, 13] In 1978 an encapsulated form of the enzyme, Esperase (Novo Industries, Copenhagen, Denmark), one of the *Bacillus*-derived exoenzymes, had been introduced into the dry bleach manufacturing process at this factory. The Esperase was manufactured by one of the most current technologies for production of enzyme granulates. Bacterial enzymes had not been previously used in the plant in any form, unencapsulated or encapsulated. The aim of this study was to determine whether or not workers exposed to marumed enzyme had developed immunologic reactivity or subsequent clinical sensitivity.

METHODS

Dry bleach manufacturing process

The manufacturing process is illustrated in Fig. 1. The dry-enzyme concentrate (8% to 10% Esperase by weight) is supplied in an 1800-pound bag, which is suspended above a weigh hopper. The enzyme is dispensed to the weigh hopper by gravity and is metered automatically to a force-feed assembly unit via a time-operated addition screw. Soda ash and sodium perborate (two primary components) are stored in silos on the plant roof and are added into the ready weigh hopper and force-feed assembly by gravity. The dry bleach-enzyme mixture is then pumped into the rotating blender where it is mixed with trace additives (whiteners, brighteners, perfumes). The dry bleach is then processed through a scalping screen to achieve the proper particle size. This finished product is conveyed to a hopper that feeds lines that pack the bleach into cartons. The cartons are inspected, cased, and conveyed to a warehouse.

Environmental evaluation

Air samples for measurement of total airborne particulate and proteolytic-enzyme dust were obtained by use of high-volume samplers and high-efficiency glass fiber filters. The samplers were strategically positioned at fixed locations in the plant that were best suited to estimate exposure conditions of the employees. Samples were taken for 7½ to 8 hr at a flow rate of 36 cfm. Total particulate concentrations were determined gravimetrically and reported as micrograms of total dust per cubic meter of air ($\mu g/m^3$). Proteolytic enzyme content was determined by means of a modification of the N, N-dimethylcasein method.[14, 15] Enzyme concentrations were reported as micrograms of proteolytic activity per cubic meter of air ($\mu g/m^3$) on the basis of a predetermined assay that Esperase enzyme contained 2.6% pure crystalline-proteolytic enzyme.

The recommended level of exposure to Esperase was calculated from the total enzyme dust TLV of 0.06 $\mu g/m^3$ proposed by the ACGIH.[16] The calculated enzyme-equivalent expression for Esperase was 3.9 $\mu g/m^3$.[12]

The size-mass distributions of airborne dust particles were determined by use of a 1 cfm eight-stage cascade impactor. The impactor was positioned adjacent to the high-volume samplers; sampling periods were the same as for the high-volume samplers.

Eight hour time-weighted average personal breathing-zone exposures to total particulates were determined by use of standard sampling techniques. Particulates were collected at a flow rate of 0.07 cfm on 37 mm diameter, 0.8 μm pore size, polyvinyl chloride membrane filters that were contained in three-piece closed face cassettes. Total particulate concentrations were determined gravimetrically and reported as micrograms per cubic meter.

Medical evaluation

We evaluated 13 employees who were regularly exposed to enzyme dust (representing 93% of the exposed group), two employees exposed in the past, and nine employees who worked in adjacent buildings without any direct history

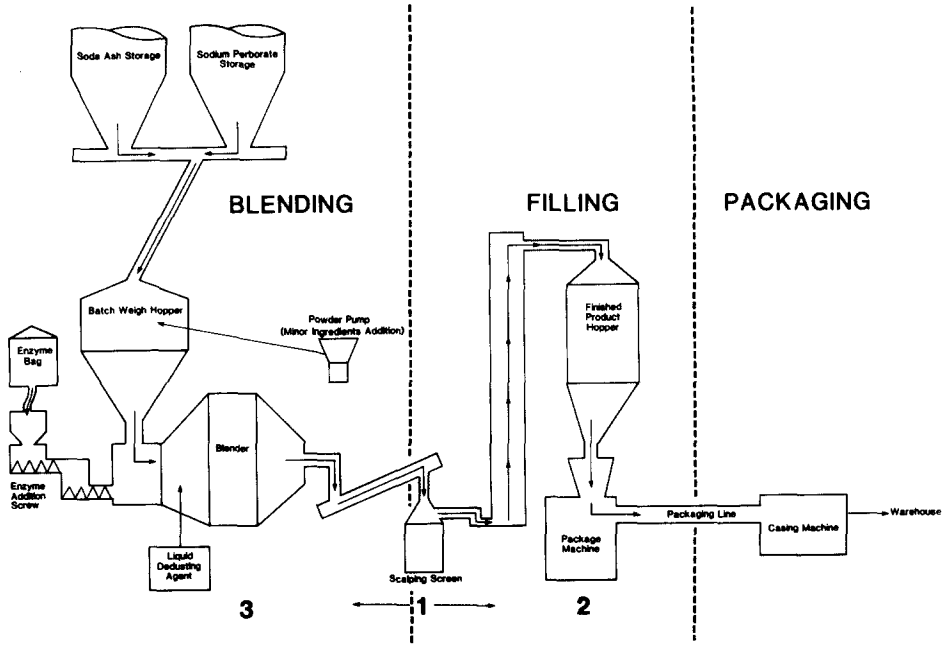

FIG. 1. Schematic of dry bleach manufacturing process. Work positions for line mechanic, filler operator, and blender operator are indicated by numerals 1, 2, and 3, respectively.

TABLE I. Concentrations of total and enzyme-specific airborne dust in high-exposure areas in an enzyme bleach plant

Sample location	8-hr Time-weighted average airborne concentration ($\mu g/m^3$)	
	Total dust	Enzyme-specific dust
Blending area	410 ± 180*†	0.64 ± 0.61
Filling area	380 ± 240‡	0.49 ± 0.47

*Mean ± SD.
†N = 8.
‡N = 7.

of exposure. The enzyme had been introduced to the plant 2 yr before the study. The average duration of employment among the exposed workers since introduction of the enzymes was approximately 17 mo with a range of 1 mo to 2 yr. The two previously exposed employees were transferred for nonmedical reasons. One had worked on the production line for 15 mo, and the other one had worked for 5 mo with cessation of exposure 6 and 2 mo, respectively, before the study.

Clinical evaluation of all study participants included: (1) an American Thoracic Society respiratory questionnaire that was administered by experienced interviewers and modified to obtain data on past history, jobs within the plant, and skin complaints, (2) a limited physical examination, and (3) pulmonary-function testing by use of an Ohio Medical dry-rolling seal spirometer. These tests were performed both before and after shift in enzyme-exposed employees and once during the shift in nonexposed employees. Because skin testing was not possible in this study, atopic status was assessed by evaluation of questionnaire responses regarding the personal and family prevalence of the common allergic diseases (hay fever, allergic asthma, eczema); these data were evaluated by examiners who were blind to exposure or serologic data.

Immunologic evaluation

Serum samples were obtained and tested in triplicate for measurement of specific-IgE antibody to the Esperase-bacterial enzyme by use of RAST. Pure crystalline-Esperase enzyme that was obtained from the manufacturer (Novo Industries) was coupled to methycellulose discs that were previously activated with cyanogen bromide[17] by incubating activated discs with 3 ml of a 20 mg/ml enzyme solution. Subsequent steps of the RAST were then performed according to standard methodology that was previously reported by this laboratory.[18] A positive result was defined as percent binding greater than twice that of four nonallergic laboratory personnel who had not been exposed to Esperase.

Specificity of the IgE-antibody response to Esperase was verified by RAST-inhibition tests.[18] All enzyme absorbents that were used in this technique were inactivated by PSF.[19] The inhibition caused by the PSF-inactivated Esperase was compared to inhibition caused by PSF-inactivated enzyme preparations of subtilisin BPN, subtilopeptidase-A, and papaya protease that were purchased from Sigma Chemical Co., St. Louis, Mo. These inhibition studies were repeated five separate times with the worker's serum that showed the

highest RAST titer (8% binding) to Esperase. Results of RAST-inhibition experiments were expressed as the molar concentrations of respective PSF-inactivated enzymes producing 50% inhibition of IgE-antibody binding to Esperase-coupled methylcellulose discs.

Specific IgG to Esperase was determined by a modification of the ELISA method as described by Bernstein et al.[20] Aliquots of 0.2 ml of Esperase at a concentration of 200 μg/ml in 0.1N $NaHCO_3$ were placed in each cuvette of the Gilford cuvette-pak (polystyrene acrylic copolymer) and incubated stepwise at 25° C for 1 hr and at 4° C for 24 hr. Each individual cuvette was then washed three times with PBS, and a similar wash was repeated between all subsequent steps. Next, 0.2 ml samples of serum, diluted 1:10 and 1:100 in PBS with 5.0% BSA, were added and incubated for 1 hr at 25° C. To measure specific binding of IgG, 0.2 ml portions of goat anti-human IgG alkaline phosphatase conjugates (Sigma), diluted 1:1000 in PBS with 1% BSA, were added to the cuvettes for 1 hr at 25° C. Finally, 0.2 ml of 0.006M p-Nitrophenyl phosphatase, disodium (Sigma) substrate solution, diluted in alkaline glycine buffer solution (0.05M glycine and 0.5 mM magnesium chloride, pH 10.4) was added. A yellow-color reaction was observed after 10 min. The reaction was terminated at this time with 0.05 ml of 2M NaOH. OD at 405 mμ was read on a Gilford PR-50 EIA analyzer. A positive-IgG ELISA was defined as an OD 405 mμ reading twice that of four nonallergic laboratory personnel who had never been exposed to Esperase.

Total IgE was determined by radioimmunoassay with Clinical Assays Gamma Dab M ^{125}I-labelled IgE kit (Clinical Assays, Cambridge, Mass.). Results were reported in IU/ml.

Statistical analysis

Differences in spirometry between exposure groups were compared by use of a nonpaired t test. Before and after shift changes in spirometry among the exposed workers were compared by use of a paired t test. The prevalence of symptoms and positive immunologic test results between groups was compared by Fisher's exact test.

RESULTS

Environmental evaluation

Area sampling revealed that air concentrations of proteolytic-enzyme dust ranged from nondetected (<0.002 μg/m^3) to 1.57 μg/m^3 in the blending area and ranged from <0.002 to 0.76 μg/m^3 in the filling area (Table I). None of these observed levels exceeded the calculated enzyme-exposure standard of 3.9 μg/m^3. The corresponding total particulate concentrations ranged from 180 to 870 μg/m^3 (Table I). The Soap and Detergent Industry Association uses a 1000 μg/m^3 (1 mg/m^3) particulate level as a guideline for controlling workplace dust containing proteolytic enzymes. The aerodynamic particle-size distributions

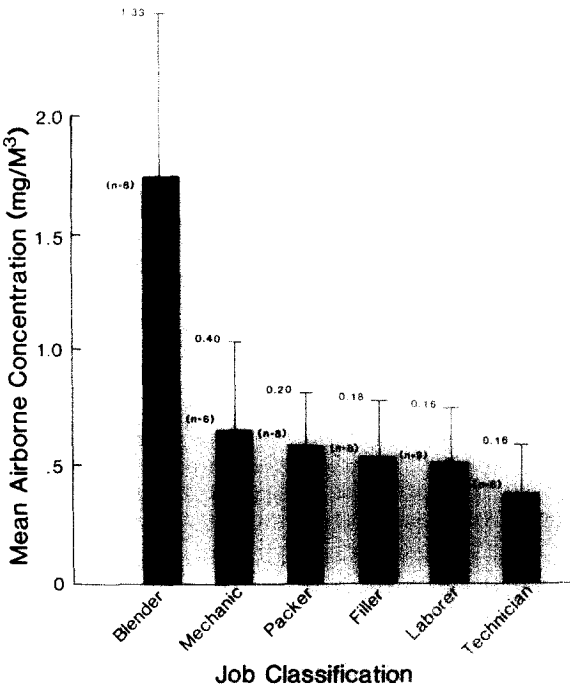

FIG. 2. Personal breathing-zone exposure to total particulates (mean ± S.D.) by job classification for enzyme bleach workers.

indicated that approximately 50% of the particulates were within the respirable range (<5 μm) in both blending (4.8 μm ± 4.1, mass median diameter ± geometric standard deviation) and filling (4.0 μm ± 5.4) areas.

Personal breathing-zone exposures to total particulates are listed in Fig. 2. The blender operator demonstrated highest exposure, followed by line mechanic, package inspector, laborer, and quality control technician. Thirteen percent (6/45) of the measurements exceeded the 1 mg/m^3 total dust level used by the detergent industry as an engineering safety guideline. Area sampling data demonstrated lower airborne-dust levels than did the breathing-zone samplers because the area-sampling technique could not account for the movement of workers from one area to another nor for occasional acute high exposure that resulted from process-equipment failure. Therefore the area measurements should be considered to represent minimal exposures. In general, respiratory protection was not worn by workers.

Medical evaluation

A total of 24 employees were interviewed and examined. Significant clinical factors that were unique to these 24 workers are shown in Table II. Of the 13 exposed workers, 12 (92%) were smokers, whereas

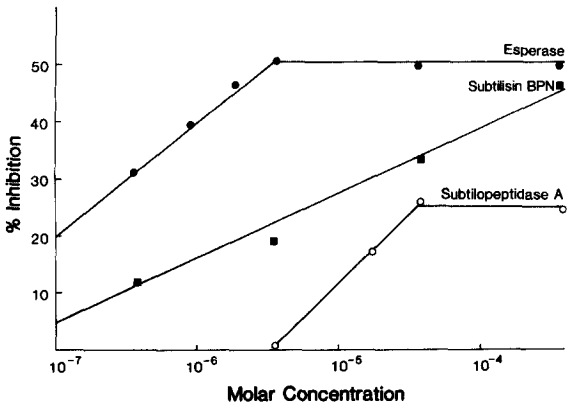

FIG. 3. RAST-inhibition experiments with Esperase-sensitive serum. Each curve was plotted from dose–response RAST assays of serum obtained after absorption with each of the designated PSF-inactivated enzymes.

seven (77%) of the nine nonexposed were smokers. Historical information for six of the 13 currently exposed workers and for one of the past exposed workers suggested an atopic background. Two (22%) of nine nonexposed workers were atopic.

Analysis of questionnaire data revealed upper and lower respiratory symptoms to be equally common in both exposed and nonexposed groups. Among the 13 currently exposed employees, six had one or more eye, nose, throat, or chest symptoms that appeared to be occupationally related and that had appeared only after introduction of the enzymes, six workers had no such symptoms, and in one the questionnaire responses were considered equivocal. Among the nine nonexposed workers, four had one or more respiratory symptoms, whereas five had none. The two workers with past exposure were asymptomatic. The prevalence of auscultatory wheezing after a work shift (two versus one) and evidence of skin rash (three versus one) was similar in exposed and nonexposed groups.

Pulmonary-function tests were performed before and after a work shift in the exposed workers but only once during the shift in nonexposed workers. There was no overall difference in baseline spirometry between the exposed and nonexposed workers. However, among the exposed workers there was a significant decrease over the work shift in FEV_1 (mean decrease of 0.114 L; $p < 0.05$).

Immunologic evaluation

Results of RAST (Table III) were evaluated by job classification and in relation to enzyme-dust exposure. Positive Esperase–specific IgE RAST (8.0%, 4.4%, and 3.4% binding) were obtained in three (no. 3, no. 1, and no. 6, Table III) of the 12 exposed

TABLE II. Summary of significant clinical factors in workers exposed to enzyme dust

	Currently exposed	Exposed in past	Non-exposed
Number	13	2	9
M/F	9/4	1/1	6/3
Mean age (yr)	37.5	38	38
(Range)	(21 to 56)	(27 to 49)	(25 to 54)
Cigarette smokers	12 (92%)	0	7 (77.8%)
Positive atopic status*	6	1	2

* As assessed by questionnaire responses regarding personal and family prevalence of the common allergic diseases.

workers from whom blood samples were obtained. None of the 11 currently nonexposed workers had significant RAST binding (range 0.6% to 1.4%). The three exposed workers (blender, mechanic, filler) with positive RAST were exposed to relatively high dust levels as determined by personal breathing–zone sampling data (Fig. 2). The work positions for the RAST positive line mechanic, filler operator, and blender operator are indicated by numerals 1, 2, and 3, respectively, in Fig. 1. Of these three the mechanic had eye and chest symptoms that appeared to be occupational in origin because the onset had occurred after the introduction of the enzymes, the filler operator had symptoms that were considered equivocal, and the blender operator reported no symptoms. Two of three workers with positive RAST were considered to be atopic on the bases of their past medical histories, whereas three (33%) of the nine RAST–negative exposed workers were considered atopic. Four (44%) of these nine were asymptomatic, whereas five were symptomatic. The before and after shift change in FEV_1 was similar in RAST-positive workers (mean decrease of 0.130 L) and RAST-negative workers (mean decrease of 0.108 L).

Levels of IgE in currently exposed and RAST-positive workers ranged from 110 to 125 U/ml. Workers with positive-Esperase RASTs of 8%, 4.4%, and 3.4% had IgE levels of 110, 112, and 125 U/ml, respectively. These values were similar to IgE levels in nonexposed workers ($\bar{x} = 66.2$, range < 5 to 175 U/ml).

The results of inhibition studies demonstrated the immunologic specificity of the Esperase RAST (Fig. 3). Fifty percent inhibition of binding was achieved by an average concentration of 3×10^{-6}M Esperase. Subtilisin BPN inhibited the RAST by 47%, but the concentration that was required for this inhibitory effect was almost two logs greater than the Esperase-

TABLE III. Summary of esperase-specific RAST and ELISA results in workers exposed to enzyme dust

Workers	Job description	Atopic status	Esperase-specific antibody	
			IgE (RAST) % Binding	IgG (ELISA) OD 405 mμ
Current exposure (13)*				
1	Blender	A	4.4†	0.40
2	Blender	NA	1.3	0.12
3	Mechanic	A	8.0†	0.82†
4	Mechanic	NA	1.2	0.56†
5	Packer	NA	1.1	2.41†
6	Filler	NA	3.4†	2.39†
7	Filler	NA	0.8	0.23
8	Laborer	A	1.0	0.18
9	Laborer	NA	1.2	0.35
10	Laborer	A	1.1	0.15
11	Technician	NA	1.6	0.24
12	Technician	A	1.3	0.24
Previous exposure (2)				
13	Packer	NA	1.4	2.06†
14	Laborer	A	1.3	0.33
Nonexposed (9)	Shipping		0.6 to 1.4 $\bar{x}$ = 1.0	0.09 to 0.45 $\bar{x}$ = 0.21
Negative controls (4)			1.1 to 1.5 $\bar{x}$ = 1.3	0.15 to 0.25 $\bar{x}$ = 0.21

A = atopic; NA = nonatopic; $\bar{x}$ = mean.
*Serum not available in one worker.
†Significant values are defined as twice that nonexposed laboratory workers.

inhibiting concentration. The degree of inhibition caused by subtilopeptidase-A was probably not significant. Papaya protease and ragweed controls did not exhibit inhibitory properties.

Results of Esperase–ELISA specific IgG testing are shown in Table III. These tests were obtained with serum dilutions of 1:10. Dilutions higher than 1:10 were below detection limits of this Esperase-assay system for all of the samples tested. Four (33%) of the currently exposed workers had positive Esperase–specific IgG ELISA tests; two of these current workers also had Esperase–specific positive RAST. These employees, a filler operator, a package inspector, and two line mechanics, held jobs with high indices of exposure (Fig. 2). One of the previously exposed workers who had a negative RAST had an IgG-ELISA result 10 times higher than the control mean value. This previously exposed employee with specific-Esperase IgG had been exposed to Esperase during work as a package inspector for 15 mo, but this exposure had ceased 6 mo before the study. None of the nonexposed workers had significant–IgG ELISA titers. Of the two workers with both specific IgE- and IgG-positive tests, occupationally related symptoms were present in one and equivocal in the other. The package inspector and mechanic with Esperase specific–IgG positive and IgE-negative results were both asymptomatic. Only one of the five workers with significant titers of IgG was considered to be atopic.

Table IV illustrates that there was an association between positive antibody reactions (specific IgG, IgE, or both) and exposure to the enzyme detergent. This association was statistically significant ($p < 0.05$).

DISCUSSION

Past studies[2–7] have shown that some factory workers who were exposed to bacterial enzyme dust developed both upper and lower respiratory symptoms that ranged from nasal stuffiness, rhinorrhea, lacrimation, and throat irritation to chest tightness, cough, and breathlessness. Positive skin tests, presence of specific-IgE antibodies, and positive-challenge tests demonstrated an immunologic mechanism in the etiology of these symptoms. Soon after the occupational problems that were associated with bacterial enzyme detergents were first reported, claims were made that decrease of dust-exposure levels would greatly reduce, if not eliminate, future health problems in this industry.[1, 6, 7]

The results of the present cross-sectional analysis of a limited number of workers at a dry bleach plant

TABLE IV. Comparison of antibody test results (Specific IgE, IgG) with exposure status in workers exposed to enzyme dust

Test results	Exposure status		
	Exposed	Never exposed	Total
One or both tests positive	6	0	6
Neither test positive	8	9	17
	14	9	23

$p = 0.0297$.

revealed that there are still exposures in the workplace that are sufficient to cause IgE–mediated immunologic sensitization in 25% of exposed workers. This situation prevailed even though exposure was limited to "nonrespirable" enzyme particles that were produced by a process wherein the enzyme was encapsulated, "spheroidized," or "marumerized" into pellets. Evidence of sensitization was obtained even though the dust level of enzymes recorded in this survey was below the calculated ACGIH–TLV enzyme equivalency of 3.9 $\mu m/m^3$. However, it is possible that peak levels or accidental spills that were undetected in this cross-sectional survey could have played a substantive role in the induction of sensitivity in some of these workers. Since it had been previously demonstrated that a positive RAST has a significant correlation with positive bronchoprovocation and/or clinical sensitivity, three workers who demonstrated Esperase-specific IgE in this study must be considered at high risk for present or future clinical sensitivity.

A set of guidelines that has already been successfully initiated by the Detergent and Soap Industry for the enzyme encapsulation process uses a limit of 1 mg/m^3 for total dust-area samples. The results of area samples that were obtained in this study did not exceed this 1 mg/m^3 criterion. However, a number of personal samples that may more accurately reflect actual worker–breathing zone exposure did exceed it (Fig. 2). Thus despite the use in the study plant of a dust–control engineering system and a modern encapsulation process, our data indicate that these actions did not prevent some of the workers from developing humoral-IgE sensitization.

Since direct skin or provocation testing was not permitted in this survey, "sensitization" of plant employees was indexed by the presence of specific–IgE humoral antibodies as detected by the RAST. Three workers who were currently exposed to the highest levels of Esperase dust (Fig. 2) had significant levels of Esperase-specific IgE, whereas employees with previous or no exposure to Esperase had RAST values comparable to the negative controls. The range of total IgE levels in currently exposed workers was similar to the range in nonexposed workers and well within the limits of normal IgE values in our laboratory. This indicates that specific-IgE data were not dependent upon total IgE and that the latter test would not be a useful predictor of Esperase sensitization.

Specificity of the RAST was verified by inhibition studies. It should be noted that the peak level of inhibition for the homologous enzyme was 50%. This was a lower inhibitory activity than has been obtained with RAST–inhibition studies by use of inhalant allergens, which usually inhibited as much as 90%.[18] This discrepancy may be due partially to chemical inactivation of the enzyme-inhibitory reagents. Although inactivation is required to avoid nonspecific reactions with test sera, it nevertheless may destroy some adjacent allergenic determinants on the enzyme molecule. The same rationale may also explain inhibition results that are obtained with the heterologous-enzyme reactant, subtilisin BPN (Sigma Chemical Co.). Despite these shortcomings, the specificity requirements for demonstration of RAST inhibition were satisfactorily fulfilled.

Esperase-specific IgG, as measured by the ELISA technique, also appeared to depend upon exposure patterns. Employees who were exposed to higher dust levels had significant levels of Esperase-specific IgG (Table III). In contrast to the RAST results, one of the previously exposed employees had IgG. This finding appeared to be consistent with his relatively long past exposure to the enzyme. Nonexposed workers did not demonstrate specific-IgG antibody.

As determined by the health questionnaire survey, the frequency of upper and lower respiratory symptoms was similar in the exposed and nonexposed workers. This lack of intergroup discrimination suggests a limitation of the questionnaire that was used in this particular study. Without the opportunity for additional personal interviews, skin tests, or serial prospective studies, it was not possible to conclude whether there was an excess prevalence of symptoms that was attributable to enzyme-dust exposure in this relatively small group of workers. However, the evidence of serologic "sensitization" that was obtained in this study would suggest that the workers with positive Esperase-specific IgE are at risk for developing future clinical sensitivity, and that all exposed workers should have serial determinations of specific IgE in order to detect early evidence of sensitization. The causes of the respiratory symptoms in the nonexposed workers are not apparent. These may have been related to smoking or to possible exposure to other irri-

tant materials, such as chlorine and alkaline dust that were both present in the plant.

In previous reports of sensitization that is induced by uncapsulated enzyme preparations, preexisting atopic susceptibility occurred more often in clinically sensitive workers. The current study population was too small to determine whether previous atopic status predisposed to the development of Esperase-specific IgE. Two of three workers with Esperase–specific IgE reactions had a history of family or personal allergic disease, but this type of history was also obtained in three out of nine workers with negative RAST to Esperase.

Although a significant decrease in FEV_1 among exposed workers over a work shift occurred, no definite conclusions can be inferred from these limited data inasmuch as it was not possible to test nonexposed subjects before and after the work shift. This was an obvious handicap of the cross-sectional study.

There was an association noted between positivity for one or both tests of specific antibody and exposure to the enzyme detergent (Table IV). Thus the RAST and ELISA, although not quantitative techniques, appear to detect specific immunologic reactivity to Esperase and can be used as screening tests. We cannot predict the future clinical course of those immunologically sensitized workers without serial studies. The RAST and ELISA may be useful methods of identifying workers whose exposure to enzyme dust may be potentially hazardous despite the technologic improvement of marumerization. Such workers, identified by positive RAST and/or ELISA, should be evaluated serially by quantitative immunoassays, skin testing, and more detailed clinical evaluation by an experienced physician who is familiar with occupationally induced allergic disease.

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