

Enzyme allergy in populations exposed to long-term, low-level concentrations of household laundry products

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Twenty-five per cent of 353 highly and moderately sensitive patients attending allergy clinics in Cincinnati and Middletown, Ohio, exhibited positive skin tests to either one or both of the 2 major types of commercial detergent enzymes, Alcalase (ALC) and Amylase Protease (AP). Positive passive transfer results were obtained in 5 of 10 patients who exhibited direct skin reactivity. Nasal and bronchial challenge tests were performed in 14 patients, all of whom related the onset of respiratory symptoms to prior exposure to enzyme-containing products. Positive nasal tests were observed in 7 patients with histories of upper respiratory symptoms, and positive bronchial responses were obtained in 7 patients with antecedent complaints of lower airways obstruction. Eleven of these patients were atopic; 3 were nonatopic. All patients noted significant improvement in causally related symptoms after enzyme products were eliminated.

The occurrence of occupational sensitization to enzyme dust generated during the production of detergents has been documented in many reports.¹⁻¹² To date, 482 workers have exhibited a common pattern of immediate and late respiratory symptoms after exposure, positive immediate skin tests, and functional evidence of airways obstructive disease. In some cases, transient impairment of the carbon monoxide gas transfer factor was observed.^{6, 10} Sensitization developed in both atopic and nonatopic individuals, although the incidence rate apparently favors the former group. It was further established that the rate of appearance of occupational sensitization could be correlated with degree of exposure to high enzyme dust concentrations in the plant environment. Indeed, it was possible to reduce the number of new cases by instituting improved dust control measures. Since levels of dust containing these enzyme additives in the household environment are much less than those in the factory situation, industry scientists predicted that consumer sensitization would not be possible.^{13, 14} The paucity of causally related consumer complaints, despite extensive use of enzyme-containing detergents since 1964 in Europe and 1967

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in the United States, appears to support their contention. Nevertheless, when faced with the facts that 2.5 million metric tons of detergents are used in the United States annually¹⁵ and that until recently 80 per cent of these products contained enzyme additives, the importance of determining potential sensitization in the consumer population is self-evident.

In 1970, a preliminary report suggested that allergic symptoms developed in individuals exposed to long-term, low-level concentrations of enzyme laundry products.¹⁶ Symptoms, skin reactivity, and provocative testing in this group of people corresponded to clinical data previously reported in occupational cases. Of further interest was the recent report of Belin and co-workers.¹⁷ They demonstrated positive skin tests to Alcalase enzyme by the prick method at a dose of 1 μ g of protein per milliliter, specific IgE antibodies, and clinical symptoms in 3 housewives, one with asthma and 2 with rhinitis and eye symptoms. Two additional cases in consumers have also been reported.^{7, 18} This is a report of additional consumer studies in which the clinical techniques of skin testing, passive transfer tests, and nasal and bronchial challenge methods were employed in a population attending allergy clinics for diagnostic evaluation and in several patients who were able to relate their symptoms to prior exposure to enzyme-containing laundry products.

METHODS AND MATERIALS

Patients

Clinical studies were divided into two general categories: (1) a survey of skin sensitivity in groups of patients undergoing diagnostic evaluation in two allergy clinics located in Cincinnati (Group 1) and Middletown (Group 2), Ohio, and (2) skin, nasal, and bronchial studies in 14 patients who had recently developed respiratory symptoms and from whom a detailed history revealed a reasonable suspicion of onset of symptoms following exposure to enzyme-containing detergents.

Group 1 consisted of 461 patients from within a 15 mile radius of Cincinnati, Ohio, and Group 2 included 78 patients residing 30 miles from Cincinnati. The sex distribution of Groups 1 and 2 was 232 men and 307 women. Ages in these combined groups ranged from 16 to 66 with a mean of 29 years.

Materials

Two enzyme products, Alcalase (manufactured by Novo Industri A/S, Copenhagen, Denmark) and Amylase Protease (supplied by Monsanto Chemical Corp., St. Louis, Mo.), commonly used in detergent formulations were kindly provided by a major soap and detergent producer. These materials were supplied as dry powders containing inorganic salts, a few viable spores, and about 35 per cent organic matter of which the total enzyme content is estimated to be 5 to 10 per cent.* Stock solutions of Alcalase (ALC) were prepared in phosphate-buffered saline (pH 7.2) at a concentration of 10 mg. per milliliter. Solutions of Amylase Protease (AP) were made similarly at a concentration of 3.75 mg. per milliliter. Protein concentrations determined by the method of Lowry and associates¹⁹ were 2.5 mg. per milliliter and 1.9 mg. per milliliter for ALC and AP, respectively.

*The enzymatic activity in each major type (Alcalase and Amylase Protease) of crude enzyme concentrate is estimated to be about 4 per cent of the proteolytic activity of the purified alkaline proteases derived from *B. subtilis*, subtilopeptidase A and B. Alcalase is essentially identical to subtilopeptidase A, while the alkaline proteinase in Amylase Protease corresponds to subtilopeptidase B.

Sterilization was accomplished by passage through 0.45 μ Millipore membranes. Fresh stock solutions were prepared every 4 weeks, although it had been previously determined that these extracts retained their skin reactivity for as long as 6 months when stored at 4° C. The enzymatic activity of stock solutions disappears rapidly. A 1:100 dilution of the enzyme stocks in the same buffer was made each week for all skin-testing and organ challenge procedures. After ascertainment that heat treatment of stock solutions at 100° C. for 30 minutes did not alter their skin reactivity,* bronchial and nasal provocations were performed with heated solutions beginning July 1, 1970. Histamine content was assayed on fresh and 5-month-old stock solutions of ALC and AP, according to a modification of the method of Shore and associates.²⁰ Fresh stock extract values, 0.005 μ g per milliliter and 0.002 μ g per milliliter, respectively, were approximately 1,000-fold less than the dose of histamine required for a skin-whealing response. There were no appreciable changes in the histamine content of solutions stored for 5 months.

Since bovine ribonuclease and papain had been utilized previously in occupational skin surveys as reference controls, they were also included in the present investigations. These substances were obtained from commercial sources† and were processed in the same manner as described above. However, stock solutions of these enzymes were made in a concentration of 1.5 mg. per milliliter for several reasons. Salvaggio and associates²¹ had previously demonstrated that this stock concentration of bovine ribonuclease was satisfactory for clinical studies. Second, the commercial samples of bovine ribonuclease and papain actually contained more active enzyme than the "as received" enzyme powders used in detergent manufacturing. Thus the protein concentration of the stock bovine ribonuclease solution was 0.4 mg. per milliliter, an amount approximately equivalent to the enzyme protein calculated to be present in the crude extracts of ALC and AP. This relationship did not hold for the stock papain solution, the protein concentration of which was only 0.3 μ g per milliliter. It was suspected, though not proved, that rapid autolytic activity of the papain solution was responsible for the reduced protein content of this antigen.

Allergenic extracts of pollens, molds, and dust were obtained commercially. Testing solutions of pollens and molds were 1:1,000 (500 PNU per milliliter), while the testing solution of Endo dust was 1:10,000.

Scratch tests were performed with ALC and AP enzyme extracts at concentrations of 10 mg. per milliliter (1:100) and 3.75 mg. per milliliter (1:375), respectively. Intracutaneous tests were done at concentrations of 0.1 mg. per milliliter (1:10,000) ALC and 0.0375 mg. per milliliter (1:37,500) AP. In previous studies it had been determined that these concentrations elicited only rare irritant-type reactions. The test solutions were injected intracutaneously in 0.02 ml. quantities on the volar aspect of the forearm, and results were interpreted in 15 minutes. Phosphate-buffered saline (pH 7.2), the diluent employed for the enzyme solutions, was injected into control sites. Reactions to test extracts were considered positive if the scratch tests showed significant wheal and flare or if the intracutaneous response was judged to be 2+ or greater.‡

Passive transfer studies

Passive transfer studies were performed on the backs of 2 normal volunteer subjects who had no history of prior exposure to enzyme dust. Skin sites were prepared by intracutaneous injections of varying dilutions of fresh, unheated sera. One site was pre-

*Enzymatic activity is completely destroyed by this treatment.

†Nutritional Biochemicals, Cleveland, Ohio.

‡Criteria for positive scratch tests read after 20 minutes: 2+—a wheal (with or without erythema) 5 mm. in diameter greater than phosphate-buffered saline-glycerol control; 3+—a wheal (with or without erythema) 6 to 10 mm. in diameter greater than control; 4+—any wheal with pseudopodia. Criteria for positive intracutaneous tests read after 15 minutes: 2+—a wheal 3 $\times$ the size of control (with or without erythema); 3+—a wheal 4 $\times$ the size of control (with or without erythema); 4+—any wheal with pseudopodia.

pared with undiluted serum heated at 56° C. for 30 minutes. Forty-eight hours later these sites were challenged with 0.02 ml. quantities of 1.0 mg. per milliliter enzyme solution (1:1,000). Appropriate controls included phosphate-buffered saline (pH 7.2) and 1:1,000 dilutions of the unrelated enzymes, papain, and bovine ribonuclease. Additional controls consisted of skin tests with all the above substances in areas of skin not previously injected with test sera.

Organ challenge tests

Nasal. All patients were asymptomatic on the day of test as determined by base-line rhinoscopy and, in some cases, by objective measurements recorded by the rhinorheometer,* an updated model of the Butler-Ivy apparatus, which measures nasal airways resistance under conditions of preset air flow of 6.8 L. per minute.²² Nasal challenge tests were accomplished by aerosolizing 0.5 ml. of test solutions via a No. 40 DeVilbiss nebulizer with a special nasal attachment. Preliminary control tests with isotonic saline showed no change from base-line observations in all cases. Physical examination, subjective response, and objective studies (when included) were recorded at 10 minute intervals for 60 minutes.

Bronchial. Challenge tests were performed when patients were asymptomatic. Control and test solutions were self-administered through a No. 40 DeVilbiss nebulizer by a hand bulb for 5 minutes at the patient's usual respiratory rate. Following base-line chest examination and determination of the mean of 3 peak expiratory flow rate† maneuvers, isotonic saline was nebulized. All studies were repeated 10 minutes later, after which tests with serial concentrations of enzyme solutions were begun. Results were recorded every 10 minutes. In one patient, results were also obtained 6 hours after the provocative episode. The protocol for spirometric studies was based on methods outlined by Kory and associates.²³ Gas transfer factor was estimated by means of the single-breath carbon monoxide test.²⁴

Test solutions for both bronchial and nasal challenge tests ranged from 1:100,000 to 1:1,000, the latter being utilized only after previous dilutions had yielded negative responses. Control bronchial provocations in 5 nonsensitive volunteers had established that 1:1,000 unheated solutions of these antigens were not primary irritants.⁶ Primary irritant nasal tests performed in 3 other normal volunteers were also negative. Because patients with asthma and rhinitis may respond nonspecifically to low levels of irritants in aerosolized solutions, nasal and bronchial provocation tests were also administered to 5 patients with rhinitis and 5 patients with asthma, all of whom were judged to be nonsensitive to enzyme additives on the basis of history and skin test data. None of these patients demonstrated positive immediate or late responses after exposure to 1:1,000 unheated subtilisin solutions. Inasmuch as detailed respiratory irritant threshold experiments with bovine ribonuclease and papain were not available, these enzyme solutions were not utilized in provocative testing.

RESULTS

Skin test results obtained in Group 1 patients are shown in Table I. Patients were divided into high, moderate, and low reactive categories on the basis of personal and family history of allergic diseases and their prior skin test results to a battery of common inhalant allergens. In the highly reactive group, a significant percentage of positive reactions to all enzyme antigens was observed. The percentage of intracutaneous reactions to enzyme extracts was about the same as reactivity to individual pollen and mold allergens in

*Obtained from Vick Divisions Research Co., Mount Vernon, N. Y.

†These measurements were performed with a Wright peak flowmeter.

TABLE I. Intracutaneous skin test survey of 461 patients in greater Cincinnati area: Number and per cent positive (2+ or >) reactions*

Enzyme antigen	Test dose concentration		Allergic classification†					
	Weight/volume (μg/0.02 ml.)	Protein (μg/0.02 ml.)	High		Moderate		Low	
			No. positive	% positive	No. positive	% positive	No. positive	% positive
Alcalase	2.0	0.5	78	27.1	5	7.7	2	1.9
Amylase Protease	0.75	0.38	84	29.2	5	7.7	2	1.9
Papain	0.30	—	65	22.6	1	1.5	3	2.8
Bovine ribonuclease	0.30	0.08	73	25.3	3	4.6	1	0.9

*See text for skin test scoring criteria.

†Allergic classification: high—patients (288) with positive family and personal history of allergy; 3+ or > reaction to at least one pollen allergen or two or more nonpollen allergens; moderate—patients (65) with positive family and/or personal history of allergy; 2+ reaction to at least one pollen allergen or 3+ reaction to one nonpollen allergen; low—patients (108) with negative family and personal history of allergy; skin reactions to all allergens < 2+.

this group of patients and about 10 to 15 per cent higher than reactions to individual animal danders. Four per cent of these patients displayed positive scratch tests to the subtilisin test solutions. Scratch reactivity to the reference enzyme extracts varied between one and 2 per cent. Positive intracutaneous reactions to the subtilisin solutions were obtained in 8 per cent of the moderately reactive group and in 2 per cent of the low reactive subpopulation.

Table II summarizes skin test results in Group 2, i.e., in patients who reside in a smaller city 30 miles from Cincinnati. Although the total number of patients tested in Group 2 was much less than that in Group 1, it is noteworthy that the incidence of positive reactions to enzyme extracts was considerably less in Group 2. Positive scratch reactivity was not observed in this test population. Stratification of Groups 1 and 2 by sex revealed no significant difference. Eighty per cent of the positive reactors in both groups occurred in the first four decades of life.

To further characterize the role of skin-sensitizing antibody (SSA) in some of these patients, sera from 10 patients who exhibited either positive scratch or intracutaneous reactivity were selected for passive transfer tests. Positive transfer results were elicited in 5 instances. Control sites were negative in all patients.

Data encompassing details of exposure or causally related symptoms were not obtained in the 2 skin test survey groups. However, 7 other patients (Nos. 1, 2, 3, 4, 5, 6, 13) complained of upper and lower respiratory symptoms which appeared to correlate directly with the use of enzyme-containing laundry products. A bronchial challenge test was carried out in Patient No. 13, while nasal threshold responses were investigated in Patients 2, 3, 4, 5, and 6. All other organ provocative tests were performed in patients who demon-

TABLE II. Intracutaneous skin test survey of 78 patients in Middletown area: Number and per cent positive (2+ or >) reactions*

Enzyme antigen	Test dose concentration		Allergic classification†					
	Weight/volume (µg/0.02 ml.)	Protein (µg/0.02 ml.)	High		Moderate		Low	
			No. positive	% positive	No. positive	% positive	No. positive	% positive
Alcalase	2.0	0.5	5	12.5	1	8.3	3	11.5
Amylase	0.75	0.38	6	15	1	8.3	2	7.7
Protease								
Papain	0.30	—	5	12.5	0	0	2	7.7
Bovine ribonuclease	0.30	0.08	7	17.5	0	0	0	0

*See text for skin test scoring criteria.

†Allergic classification: high—patients (40) with positive family and personal history of allergy; 3+ or > reaction to at least one pollen allergen or two or more nonpollen allergens; moderate—patients (12) with positive family and/or personal history of allergy; 2+ reaction to at least one pollen allergen or 3+ reaction to one nonpollen allergen; low—patients (26) with negative family and personal history of allergy; skin reactions to all allergens < 2+.

strated either combined skin test and passive transfer reactivity (Nos. 8, 9, 10, and 14) or positive skin tests alone (Nos. 7, 11, and 12). Additional historical information was obtained from these subjects. Abbreviated case reports on these patients are presented below.

ABBREVIATED CASE REPORTS

Patient 1

G. B., a 31-year-old black woman, had allergic rhinitis of 5 years' duration as a primary disease. She was first observed by a physician on December 12, 1970. The seasonal pattern of the rhinitis was perennial.

She had minimal symptoms until a pregnancy in 1968. At that time she noted accentuation of nasal symptoms on days on which she did her laundry. Symptoms were always worse that evening. She also noted increase in symptoms after ironing clothes. Skin tests showed positive reactions to pollens and molds.

The enzyme-related problem was first observed by the physician also on December 12, 1970, since aggravation of nasal symptoms which had occurred appeared to be related to laundry and ironing activities.

Follow-up studies showed the patient was much improved after stopping the use of enzyme products.

Patient 2

L. R., a 34-year-old black woman, had no primary disease, and, before exposure to enzymes, she was asymptomatic with negative personal and family histories of allergy. All skin tests were negative.

An enzyme-related problem was first observed by a physician on December 5, 1970.

She had worked in a hand laundry since 1968. In November, 1970, she noted onset of severe eye and nasal symptoms during work days. Her symptoms improved on weekends, except that she also noted similar symptoms after doing her own laundry at home. She was exposed to enzyme detergents both at work and at home. Her symptoms forced her to quit the job in December, 1970, after which her symptoms disappeared completely. Because of financial need, she returned to work in January, 1971. Four days later severe

nasal symptoms recurred, and 7 days later severe asthma developed. She quit her job again within 2 weeks. She was also advised not to use enzyme products at home.

She is no longer exposed to enzyme detergents, and she is completely asymptomatic.

Patient 3

M. J., a 45-year-old white woman, had allergic rhinitis and conjunctivitis of 2 years' duration as primary diseases. The seasonal pattern was perennial, but symptoms were worse in summer months. She was first observed by a physician on August 31, 1968.

She had received immunotherapy starting in September, 1968, with marked improvement within 6 months. In February, 1971, she noted marked increase in nasal symptoms. An enzyme-related problem was first observed by a physician on February 27, 1971.

The patient had started the use of enzyme detergents in the summer of 1970. Within 3 to 4 weeks she noted itching of hands and definite increase in nasal symptoms after each load of laundry. These symptoms persisted throughout the evening.

Follow-up shows flare-up in nasal symptoms has completely subsided since elimination of products containing enzyme additives.

Patient 4

E. S., a 36-year-old white woman, had no primary disease, and her personal and family histories were negative for allergy. All skin tests were negative.

An enzyme-related problem was first observed by a physician on May 15, 1971.

Four weeks after the use of an enzyme-containing laundry product, she noted the progressive development of eye itching, lacrimation, sneezing, rhinorrhea, and nasal stenosis. Symptoms appeared first on laundry days but gradually became persistent.

Two months after discontinuation of enzyme products she reported improvement in upper respiratory symptoms.

Patient 5

P. B., a 23-year-old white woman, had allergic rhinitis and asthma, both perennial, as primary diseases. She was first observed by a physician on February 9, 1971.

Low-grade nasal symptoms had begun in April, 1969. Chest symptoms appeared in April, 1970. Skin tests were positive to pollens, molds, and dust. She has noted improvement in all symptoms since March, 1971.

An enzyme-related problem was noted by the physician at time of the first visit.

She had started using enzyme detergents in July, 1970. Within one week she noted marked increase in nasal symptoms during the laundry process. These symptoms would persist through the remainder of the day and night. She did not notice increase in chest symptoms.

After enzyme products were removed from the household, she experienced definite improvement of nasal symptoms.

Patient 6

B. G., a 28-year-old white woman with no primary disease, was first observed by a physician on March 11, 1971. Skin tests were negative. An enzyme-related problem was noted at the time of the first visit.

She had developed symptoms of chest tightness and dry cough in 1969 on days on which she used enzyme detergents. She also noted chronic redness of the eyes and rhinorrhea throughout the next two years, which was worse especially on laundry days. On two occasions she had a marked flare-up of chest symptoms. One such episode occurred after she was exposed to soap granules being used to simulate snow in a play. Chest symptoms also appeared after she handled packages in the soap section of a supermarket.

Her chest symptoms have completely disappeared since enzyme agents were eliminated. Nasal and eye symptoms have also improved.

Patient 7

E. G., a 32-year-old white woman, had allergic rhinitis which was perennial, but worse in spring and fall. She was first observed by a physician on April 20, 1971.

Her symptoms were mild until March, 1971, when she had a marked increase in symptoms. Skin tests were positive to pollens and molds.

At the time of the first visit, the physician observed an enzyme-related problem. In September, 1970, the patient had begun using an enzyme presoak. Within 2 weeks she noted severe sneezing and a sensation of chest tightness each time she used this product.

Both chest and nasal symptoms have disappeared since May, 1971, when she was advised to discontinue the use of enzyme detergents.

Patient 8

D. H., a 39-year-old black woman, had asthma of 12 years' duration, in the fall and winter months. She was first observed by a physician on September 16, 1969.

She had only mild, periodic episodes of asthma until August, 1968. She was hospitalized for asthma June, 1969, and required corticosteroids for one month. Skin tests were positive to dust, molds, and pollens.

An enzyme-related problem was first observed by a physician on January 20, 1970.

She had begun using enzyme products 9 months before severe asthma started. She noted eye, nasal, and chest symptoms on days of laundry duties.

All symptoms markedly improved after elimination enzyme detergents in February, 1970.

Patient 9

L. G., a 38-year-old black woman, had asthma of 9 years' duration. The asthma occurred in the spring and occasionally after upper respiratory infections. She was first observed by a physician on July 15, 1969.

She had mild asthma initially but had been relatively asymptomatic in the past year. Skin tests were positive to pollens and molds.

An enzyme related problem was first observed at the time of the first visit.

She had noted eye and nasal symptoms and slight chest tightness on days of laundry chores. She has been asymptomatic since she discontinued the use of these agents.

Patient 10

W. H., a 20-year-old white man, had allergic rhinitis of 9 years' duration which occurred in the spring, summer, and fall. He was first observed by a physician on July 30, 1962.

He had severe eye and nasal symptoms initially. The response to immunotherapy was good, and he was asymptomatic until 1969. He resumed treatment at that time and is again showing a good response. Skin tests were positive to molds and pollens. An enzyme-related problem was first observed by a physician on January 17, 1970.

In the summer of 1968 he had had a summer job in a soap plant. His job involved the emptying of trash cans in laboratories which handled enzyme substances. He recalled episodes of moderately severe wheezing immediately after doing this work and frequently 4 to 5 hours later.

At present he works in the mailroom of the same soap plant. He is asymptomatic except for recurrent chest symptoms after handling broken packages containing soap products.

Patient 11

M. W., a 38-year-old black woman, had asthma of 12 years' duration in late fall, winter, and early spring. She was first observed by a physician on March 4, 1971.

She had very mild symptoms until October, 1970, when her wheezing became more chronic. Skin tests were positive to pollens, dust, and molds.

An enzyme-related problem was first observed by a physician at the time of the first visit.

She had noted a marked increase in chest symptoms after using an enzyme-containing soap for washing her walls. Since then she has also noted chest symptoms on the evening of her laundry chores.

Acute episodes have subsided since she discontinued use of enzyme products.

Patient 12

C. R., a 55-year-old black woman, had had asthma for 45 years, which occurred in early and late fall and was also worse after exposure to dust, dogs, and paint. She was first observed by a physician on January 28, 1971.

She had very mild and controllable symptoms until October, 1970. Her symptoms have been chronic since that time. Skin tests were positive to ragweed pollen, molds, and dust.

An enzyme-related problem was first observed at the time of the first visit.

Since January, 1970, she had noted nasal symptoms and chest symptoms at night after using enzyme-containing detergents.

Her acute symptoms have disappeared since elimination of enzyme products. Her chronic symptoms of asthma are still present but gradually improving.

Patient 13

G. B., a 30-year-old white man, had asthma and allergic rhinitis of 2 years' duration. The seasonal pattern was perennial, but both were occasionally worse after upper respiratory infections. He was first observed by a physician on July 12, 1969.

He made an excellent clinical response to immunotherapy and was asymptomatic 6 months after this was started. Skin tests were positive to pollens, dust, and molds.

An enzyme-related problem was first observed on January 23, 1971.

He experienced a sudden flare-up of nasal and chest symptoms one week after his vacation had started early in January, 1971. During this week he helped his wife by doing laundry every day. His symptoms progressed from eye itching and nasal symptoms to severe asthma. All symptoms were aggravated when he folded freshly laundered clothes.

He has remained asymptomatic for 3 months since discontinuation of these household activities.

Patient 14

W. C., a 51-year-old black man with perennial asthma of one year's duration, was first observed by a physician on August 22, 1969.

He developed cough and wheeze one year before medical supervision. His symptoms became progressively more severe, necessitating oral corticosteroids for adequate control of symptoms. Skin tests were positive to dust, molds, and pollens.

An enzyme-related problem was first observed by a physician on January 7, 1970.

During his initial work-up he had complained of increase in cough and wheezing after using various cleaning preparations on his job. Later he noted similar symptoms after washing his socks and underwear at home. Enzyme products were used in both places.

He had not returned for follow-up evaluation in 1971.

Results of nasal provocation tests are shown in Tables IIIA and IIIB. Detailed histories in these patients revealed that upper respiratory symptoms appeared at varying intervals after repetitive exposures to enzyme-containing detergents. Positive intracutaneous tests had been demonstrated in 3 patients (Nos. 1, 4, and 7). In those patients registering negative skin tests, further testing was predicated on the basis of a strongly positive history. Positive nasal challenge

TABLE IIIA. Results of nasal challenge tests to Alcalase (ALC) and Amylase Protease (AP)

Patient No.	Type of enzyme	Test dose (mg.)	Scores of nasal changes					
			Subjective		Mean rhinoscopy		Nasal resistance (mm.)*	
			Base-line	20 minutes	Base-line	20 minutes	Base-line	10 minutes
1	ALC	0.05	1	6	1	4	—	—
2	AP	0.018	1	6	1	4	—	—
3	AP	0.18	2	6	2	4	1	50
4	ALC	0.05	1	5	1	4	1	16
5	AP	0.18	1	6	1	4	1	14
6	AP	0.018	1	6	1	4	2	7
7	AP	0.18	1	4	1	3	2	10

*Any increase from base-line value (0) of 5 mm. or more is significant.

TABLE IIIB. Description of categories for scores of nasal changes in Table IIIA.

Subjective			Rhinoscopy	
Score	Description	Definition	Parameter	Scores*
1	Nostril clear	Breathes with no difficulty through nostril	Nasal patency	1: Open
2	Nostril almost clear	Breathes easily through nostril		2: Slightly blocked
3	Nostril slightly stuffed	Breathes with slight hinderance through nostril	Nasal secretions	3: Moderately blocked
4	Nostril moderately stuffed	Breathes with difficulty through nostril		4: Closed
5	Nostril severely stuffed	Breathes minimally through nostril	Nasal pallor	1: None
6	Nostril completely stuffed	Cannot breathe through nostril		2: Slight inflammation
			Nasal edema	3: Moderate inflammation
				4: Severe inflammation
				1: None
				2: Slight
				3: Moderate
				4: Severe

*The raw scores may range from 4 to 16. Final numerical scores are determined by dividing by 4 to obtain a mean rhinoscopy score.

tests to either ALC or AP were recorded in all cases. Furthermore, positive responses corresponded to the enzyme concentrates contained in the household products to which the patient was exposed. Thus, positive tests to ALC were obtained in 2 patients (Nos. 1 and 4), while AP evoked responses in 5 patients (Nos. 2, 3, 5, 6, and 7). The challenge dose varied between 20 and 200 μ g. In several instances, challenges with both ALC and AP were performed in the same patient but on different asymptomatic days. This was done only when the first challenge series was negative to the highest concentrations tested. For example, Patient 2 did not respond after challenge with ALC, but she experienced a brisk response to AP. The responses of Patient 4 were similar, but in her case results of antigenic stimulation were reversed.

TABLE IV. Results of bronchial challenge tests to Alcalase (ALC) and Amylase Protease (AP)

Patient No.	Type of enzyme	Test dose (mg.)	Immediate symptoms*	Late symptomst	% Decrease in PEF‡	
					20 min.	6 hr.
8	AP	0.18	Marked	Marked	40	—
9	AP	0.18	Moderate	Marked	16	—
10	AP	0.018	Moderate	Marked	10	—
11	AP	0.018	Moderate	Marked	15	—
12	ALC	0.5	Indefinite	Marked	24	—
13	AP	0.018	None	Marked	15	75
14	AP	0.18	Indefinite	Marked	None	—

*Wheeze, chest tightness, or dyspnea occurring 20 minutes after challenge.

†Wheeze, chest tightness, or dyspnea occurring between 4 and 8 hours after challenge.

‡Peak expiratory flow as measured with the Wright peak flowmeter.

Table IV summarizes results of bronchial challenge tests in 6 patients with a pre-existing history of asthma and in one patient who developed wheezing only after contact with enzyme dust. Prior to skin and organ threshold tests, 4 of these patients (Nos. 8, 9, 12, and 14) were unaware of any causal relationship between exposure to enzyme additives and chest symptoms. All but one patient showed positive skin tests to one or both enzyme extracts. Sera from Patients 8, 9, 10, and 14 gave positive responses in the passive transfer tests. Following bronchial provocations, all patients experienced respiratory obstructive symptoms. Significant decrease in peak expiratory flow was noted in all 6 patients. In some instances symptoms appeared 20 minutes after the challenge dose, but it was noteworthy that all patients complained of late chest symptoms that night. Late chest symptoms were unusually severe in Patients 10, 13, and 14. Patient 13 experienced a 75 per cent decrease in peak expiratory flow 6 hours after challenge. His obstructive symptoms progressed during the following week, and he required large doses of oral corticosteroids for a period of 2 weeks. A repeat bronchial challenge study was performed on Patient 14 two months later because his first challenge test was difficult to interpret. On this occasion serial pulmonary function tests were obtained, and these results are shown in Table V. He showed a progressive decrease in ventilatory function over a 24 hour period with a return to normal in these values by 5 days. However, at this time there was a reduction in carbon monoxide gas transfer factor.

More comprehensive histories were obtained retrospectively from patients with positive respiratory challenges. In addition, their clinical courses were followed carefully after they were advised to discontinue the use of enzyme laundry products. These data are summarized in Table VI. A history of exposure to enzyme additives was obtained in all instances. The range of exposure varied between one week and 30 months. The majority of patients were classified as atopic, but 3 patients were nonatopic. It was of interest that all but one of the male patients in this group had assumed laundry chores in the household. The other male patient did have a previous industrial exposure, but this occurred in what is generally assumed to be a low exposure area. All patients noted clinical improvement after elimination of these products,

TABLE V. Serial pulmonary function changes in Patient 14 before and after bronchial challenge with Amylase Protease

Time	FEV ₁ *	FEF _{200-1,200} †	FEF ₂₅₋₇₅ %‡	DLCO§
Before	2,442	6.0	3.1	26.7
6 hr.	2,206	4.0	2.7	28.7
24 hr.	1,981	3.2	2.6	27.4
5 days	2,195	4.0	2.8	20.1

*FEV₁: forced expiratory volume (milliliters) in the first second of the forced vital capacity.

†FEF_{200-1,200}: forced expiratory flow (liters per second) between 200 and 1,200 ml. of the forced vital capacity.

‡FEF₂₅₋₇₅%; forced expiratory flow (liters per second) during the middle half of the forced vital capacity.

§DLCO: single-breath diffusing capacity for carbon monoxide (milliliters per minute per millimeters of mercury). The predicted normal value for this patient was 32.0.

but the degree of amelioration was more clear-cut in patients with no pre-existing upper respiratory symptoms.

DISCUSSION

The increased incidence of positive skin tests to the protease derivatives of *B. subtilis* in highly and moderately reactive atopic patients appears to be significant. Nevertheless, it is important to consider several relevant facts concerning the interpretation of positive, immediate skin tests in atopic individuals. It is of course essential to establish the irritant threshold of the test materials in order to exclude a direct histamine effect or some other nonspecific skin reactive factor. Both of these possibilities were excluded in preliminary experiments with the test extracts of ALC and AP.⁶ It is well recognized that highly allergic people tend to exhibit positive skin tests to a wide variety of allergens, many of which may not be related to clinical sensitivity. However, the unequivocal association of positive intracutaneous tests, positive organ responses, and clinical symptoms after exposure to enzyme products in 9 patients suggests that the testing dilutions of enzyme antigens in this study were in the range which might be expected to correlate with tissue sensitivity. But it should be emphasized that the minimal test concentration of enzyme which would detect all degrees of skin and respiratory reactivity has not yet been determined.

Intracutaneous tests proved to be very useful adjuncts in case finding, which was not always a simple task, even in the occupational milieu. Symptoms were often insidious, evanescent, and at times difficult to relate to exposure in sensitized factory workers. This difficulty may be compounded in domestic users, especially those with pre-existing respiratory disease. In addition to its value in case finding, intracutaneous testing proved to be advantageous in the epidemiologic survey, making possible the identification of the over-all potential for sensitization in the population under study. In this investigation, for example, results of intracutaneous skin testing have indicated that atopic patients possess a greater potential for sensitization to enzymes derived from *B. subtilis* and that the degree of sensitization may vary in different localities,

as evidenced by marked differences in skin reactivity between Groups 1 and 2.

The interpretation of skin testing in this study was partially restricted at the other end of the spectrum. Five patients with positive histories and provocative responses did not demonstrate positive skin tests. Negative skin tests were also encountered in about 20 to 30 per cent of symptomatic workers in two published reports.^{6, 12} Lack of skin response, therefore, does not preclude clinical sensitivity, either in workers or in consumers.

In the skin test survey, many people demonstrated high reactivity to bovine ribonuclease and papain, the unrelated enzymes used as reference controls. Positive reactions to bovine ribonuclease appear to confirm previous work by Salvaggio and associates²¹ who reported a preimmunization incidence of 20 per cent positive reactivity to similar concentrations of this antigen in atopic subjects. It is also possible that some of these positive skin tests represent cross-reactions with trace amounts of bacterial ribonuclease contained in the crude enzyme extracts.²⁵ Although reactions to papain occur less frequently, they are more difficult to explain. Preliminary irritant thresholds of this extract in atopic or nonatopic populations had not been determined in a systematic manner prior to the study, but it had been previously noted that some non-atopic workers had reacted positively to this antigen. Increased reactivity in atopic patients could reflect their heightened recognition of this antigen, which is commonly used as a meat tenderizer. It was established that heating extracts of bovine ribonuclease and papain at 100° C. for 30 minutes abolished their skin reactivity, while this procedure did not appreciably affect skin reactions to *B. subtilis* proteinases in the same patients. Because of these variables, which were not thoroughly evaluated before the skin survey was begun, it is not possible to make a direct comparison of skin reactions elicited by the reference enzymes to skin reactions caused by ALC and AP.

Induction of skin sensitivity to enzymatic metabolites of *B. subtilis* could occur in 2 ways. De novo sensitization could have occurred after suitable exposure to these materials contained in laundry products or to dried residues from laundered clothes. Since *B. subtilis* is ubiquitous in soil, positive skin reactions also could reflect prior exposure to these natural sources. The data do not exclude either possibility. Once skin sensitization is established by whatever route of exposure, it is then possible that further exposure to the proteinaceous metabolites of *B. subtilis* could have caused heightened antibody responses in these patients. Assuming that some of the allergic patients might have had pre-existing cutaneous reactivity to *B. subtilis* (before enzyme detergents were introduced into their environment), these individuals would be "primed" for an increased response upon subsequent exposure to these enzymes in the household environment. This booster effect after repetitive respiratory exposures has been demonstrated for naturally occurring ragweed allergen.^{26, 27} Moreover, previous experience gained from patients who develop spontaneous allergic or drug reactions would lead one to predict that once sensitization has occurred in a group of individuals, the dose and duration of exposure required for clinical response upon re-exposure to the specific antigen would vary widely within individuals of that group. Just this type of wide exposure

TABLE VI. Clinical summaries of symptomatic patients with histories of low-level exposure to

Patient No.	Sex	Race	Age	Allergic background		
				Family history	Personal history	Classification
1	F	B	31	+	+	Moderate
2	F	B	34	0	0	Low
3	F	W	45	+	+	High
4	F	W	36	0	0	Low
5	F	W	23	+	+	High
6	F	W	26	0	0	Low
7	F	W	32	+	+	High
8	F	B	40	+	+	High
9	F	B	39	0	+	High
10	M	W	20	+	+	High
11	F	B	38	+	+	High
12	F	B	55	+	+	High
13	M	W	31	+	+	High
14	M	B	51	+	0	High

*Symptoms: E—eye symptoms; N—nasal symptoms; A—asthma; CT—chest tightness.

†The skin test dose of ALC contained 0.08 μ g protein nitrogen (0.02 ml. of 1:10,000 extract).

‡Re-exposure: challenge—results of nasal or bronchial provocations; natural—contact with enzyme symptoms.

range was observed in patients developing symptoms after exposure to enzymes.

The organ threshold tests have provided the best evidence that clinical sensitivity has occurred in patients exposed to low concentrations of enzyme dust. Similar to the experience with occupationally sensitized individuals, both upper and lower respiratory symptoms were observed. Two patients seemed to note nasal symptoms before the appearance of lower airways obstruction. Although symptoms related to soap product exposure did not cause marked discomfort in the majority of instances, they usually persisted throughout the day and evening after exposure. Such prolongation of symptoms is usually not observed in allergic patients who may develop symptoms nonspecifically after exposure to soap powders. In all cases, a reasonable basis for a cause and effect relationship was not affirmed until the results of history, skin tests, passive transfer studies (in some), and organ challenge tests could be assessed in toto. The fulfillment of all these requirements makes case finding extremely difficult, and under these circumstances it is not surprising that more cases have not been reported. Eleven patients were classified as moderately or highly atopic, but it was interesting that 3 patients were nonatopic. In 2 patients there was limited evidence that tissue reactions to either ALC or AP were monospecific. This type of specific response may represent the clinical counterpart of the restricted cross-reactivity observed when the relatively pure subtilisins, subtilopeptidase A and subtilopeptidase B, are reacted with their respective rabbit antisera.²⁸ If these preliminary observations are confirmed, future clinical studies would be incomplete unless both major types of enzyme reagents were utilized for skin and organ testing.

All patients who experienced positive provocative tests confirmed that these induced symptoms were similar to those which appeared after natural

enzyme additives

Enzyme related data						
Exposure		Skin test reactivity†			Reexposure‡	
Months	Symptoms*	ALC	AP	Status after elimination	Challenge	Natural
24	E, N	4+	±	Improved	+	+
30	E, N, A	±	±	Asymptomatic	+	+
12	E, N	0	0	Improved	+	Not done
1	E, N	3+	2+	Improved	+	+
12	E, N, A	0	0	Improved	+	Not done
24	E, N, CT	1+	0	Improved	+	+
8	N, CT	3+	2+	Improved	+	Not done
9	E, N, A, CT	0	4+	Improved	+	Not done
20	E, N	4+	2+	Improved	+	+
24	E, N, A	4+	4+	Asymptomatic	+	+
6	E, N, A	1+	0	Improved	+	Not done
18	N, A	4+	±	Improved	+	Not done
1¼	E, N, A	2+	3+	Asymptomatic	+	Not done
12	A	0	2+	Improved	+	Not done

The skin test dose of AP contained 0.06 µg protein nitrogen (0.02 ml. of 1:37,500 extract).
detergents either deliberately or inadvertently after patient became aware of enzyme-associated

exposure. Characteristic late bronchial responses were noted in all patients undergoing bronchial provocation. The pathogenesis of these late pulmonary reactions is unknown. Pepys and co-workers² originally suggested that these delayed clinical manifestations could be the results of an Arthus reaction. However, the relationship of precipitins to this disease is as yet unresolved. For example, it has been demonstrated that the subtilopeptidase enzymes may bind nonspecifically to alpha-2 macroglobulins and alpha-1 antitrypsin serum proteins.²⁹ On the other hand, Berson and associates,³⁰ using a more sensitive radiolabeling technique, reported specific binding of enzyme antigens in the serum peak adjacent to gamma globulin. Delayed bronchospasm conceivably could also be elicited by another population of reagins analogous to different types of homocytotropic antibodies encountered in other animal species, e.g., the IgGa type in rats.^{31, 32} Parish³³ has suggested recently that a complement-dependent IgG with short-term skin-fixing properties could contribute to tissue changes in diseases of this type. Alternative nonimmunologic mechanisms have also been proposed.^{6, 34-38}

Although the association of enzyme exposure and gas transfer abnormality was documented in only one patient, it is a particularly worrisome finding. Fortunately, experience in the occupationally sensitive population indicates that this defect is reversible after the affected individual is removed from further exposure to enzyme dust.^{6, 10} However, in the case of domestic users, the possibility exists that impaired gas exchange could develop insidiously after exposure to low concentrations of enzymes over a long period of time. In such instances, an association between lung changes and exposure to enzymes might never be suspected, much less documented. Impairment of gas transfer also suggests that another mechanism might be involved. Until now

delayed hypersensitivity has not been seriously considered, but there is some evidence that this possibility should be pursued more vigorously. A typical tuberculin-like skin reaction, confirmed by skin biopsy, has been observed in one worker with occupational sensitization.¹⁶ In 1969, Simon and Pollak³⁹ reported biopsy-proved granulomatous lung disease in a worker sensitized by inhalation of glucose oxidase derived from *Aspergillus oryzae*. This organism is also a source of proteinases whose substrate specificities are very similar to those of the subtilisins.⁴⁰

Anaphylactic sensitization to a wide variety of enzymes is a calculated hazard either in occupational or laboratory settings or when these materials are used for the treatment of human disease. Apparently the anaphylactogenic potential of these agents depends upon their chemical structure, exposure dose, number of exposures, and the route of exposure. Interaction of these proteins with detergents may also affect their allergenicity, since in vitro experiments have indicated that anionic and cationic detergents are effective inducers of conformational change and helix formation in various types of macromolecular proteins.⁴¹ Whether such changes would increase or decrease the allergenic potential of enzyme molecules is unknown. Previous reports of anaphylactic reactions to trypsin and chymotrypsin are pertinent since there are many structural and chemical similarities between these enzymes of animal origin and the subtilisins used in the manufacture of soap products.⁴²⁻⁵⁰ On the basis of these common characteristics, it was predictable that anaphylactic sensitivity phenomena would appear under the proper exposure conditions. The large number of workers who developed sensitization as a result of occupational exposure has validated this assumption. As illustrated by the individual cases reported here, similar reactivity has also been demonstrated in people exposed to low-level concentrations of laundry products.

Systematic study of enzyme sensitization had not received wide attention previously because it was always much simpler to control the industrial environment or to restrict the use of enzyme products in affected patients. Crip and Beam⁵¹ reported that 14 of 94 volunteers developed positive skin tests after intramuscular injections of 10 mg. of trypsin over a 2 week period. The closest experimental corollary to the present results may be found in the experiments of Salvaggio and co-workers,²¹ in which atopic volunteers were sensitized intranasally with crystalline bovine ribonuclease. In their patients the sequence of sensitization was the appearance of direct skin reactivity followed by nasal symptoms and finally positive passive transfer tests. All three phenomena have been demonstrated in this investigation.

The soap and detergent industry maintains that consumer complaints resulting from the use of enzyme laundry products have been negligible, presumably because exposure levels under household conditions are too low. Supporting this concept was a recent industrial engineering investigation in which exposure doses were monitored under home conditions of laundry activities and in a laboratory setting in which detergents were dispensed in a like manner.¹⁴ About 0.27 μg of detergent dust exposure per cup of product was generated by ordinary consumer use, and this dose contained less than

0.05 per cent enzyme. It was concluded that not only was consumer exposure extremely low but it also was only a small fraction of levels considered safe for industrial exposure. On the other hand, guinea pig experiments conducted by Wiberg, Lavalee, and Perelmutter* under simulated conditions of household usage have demonstrated that sensitization occurs 50 to 60 days after the initial exposure. These divergent results are difficult to reconcile, especially since this investigation and 3 other reports^{7, 17, 18} have established that there is a reservoir of predisposed individuals, some of whom have already developed clinical symptoms to enzyme powders contained in detergent laundry products. Either the required sensitization and challenge doses of enzyme additives are exceedingly small or individuals are exposed to higher concentrations of enzyme dust than was detected in the industrial studies. It is also possible that consumer sensitization could have occurred by contact with dried residues of enzyme detergents on clothing, but the dosage involved in this type of exposure is unknown.

A total of 19 individuals exposed to low concentrations of these materials now have been reported to have developed hypersensitivity symptoms. This incidence of sensitivity in consumers is about 4 per cent of the total number of cases recorded in the literature thus far. The ratio of atopic to nonatopic background in the consumer group is 4:1, while it is approximately 3:2 in the occupational group.

Extrapolation of these results to the general population at this time would be premature. However, they reinforce the opinion that there is a real basis for concern regarding consumer sensitization to enzyme household products.⁵²⁻⁵⁵ Furthermore, the wide range of exposure time before symptoms develop in these patients does not permit a reasonable estimate about minimal levels of exposure which would exclude the possibility of sensitization entirely. In considering these facts, responsible officials of industry and government may exercise two options at the present time. Perhaps the more prudent decision would be to ban the incorporation of respirable enzyme materials from all soap products until the safety factor has been resolved. A second choice is under consideration by proponents of enzyme soap products. They believe that a new technology of prilling or encapsulation of detergent granules will reduce enzyme dust to a "no side effect" level. If their estimates are correct, they should be required to provide scientific data on the effects of low-level, long-term exposure to these products either by direct inhalation or indirectly from dried residues on clothing. In the meantime, labeling regulations should be invoked so that physicians may advise and consumers may choose other products if predisposing factors in a given case would increase the risk of sensitization to these substances.

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REFERENCES

- 1 Flindt, M. L. H.: Pulmonary disease due to inhalation of derivatives of *Bacillus subtilis* containing proteolytic enzyme, *Lancet* 1: 1177, 1969.
- 2 Pepys, J., Hargreave, F. E., Longbottom, J. L., et al.: Allergic reactions of the lungs to enzymes of *Bacillus subtilis*, *Lancet* 1: 1181, 1969.
- 3 Wuthrich, B., and Ott, F.: Berufsasthma Durch Proteasen in der Waschmittelindustrie, *Schweiz. Med. Wochenschr.* 99: 1584, 1969.
- 4 Newhouse, M. L., Tagg, B., Pocock, S. J., et al.: An epidemiological study of workers producing enzyme washing powders, *Lancet* 1: 689, 1970.
- 5 Greenberg, M., Milne, J. F., and Watt, A.: Survey of workers exposed to dusts containing derivatives of *Bacillus subtilis*, *Br. Med. J.* 2: 629, 1970.
- 6 Franz, T., McMurray, K. D., Brooks, S., and Bernstein, I. L.: Clinical, immunologic, and physiologic observations in factory workers exposed to *B. subtilis* enzyme dust, *J. ALLERGY* 47: 170, 1971.
- 7 Shapiro, R. S., and Eisenberg, B. C.: Sensitivity to proteolytic enzymes in laundry detergents, *J. ALLERGY* 47: 76, 1971.
- 8 McMurray, K. D.: Dermatologic and pulmonary responses in the manufacturing of detergent enzyme products, *J. Occup. Med.* 12: 416, 1970.
- 9 Slavin, R. G., and Lewis, C. R.: Enzyme asthma: An occupational disease of laundry detergent workers, *J. ALLERGY* 47: 97, 1971. (Abst.)
- 10 Nagy, S. M., Lichtenstein, L. M., Ball, W. C., et al.: Acute and chronic respiratory disease in factory workers due to the inhalation of *B. subtilis* extracts used in the manufacturing of "enzyme" detergents. Presented at the Twenty-Seventh Annual Meeting of the American Academy of Allergy, Chicago, Ill., February 20-24, 1971.
- 11 McKaba, D. G.: Skin testing as a method of detecting allergy to enzymes used in detergents. Presented at the Twenty-Seventh Annual Meeting of the Academy of Allergy, Chicago, Ill., February 20-24, 1971.
- 12 Mitchell, C. A., and Gandevia, B.: Respiratory symptoms and skin reactivity in workers exposed to proteolytic enzymes in the detergent industry, *Am. Rev. Resp. Dis.* 104: 1, 1971.
- 13 Carter, R. O., and McMurray, K. D.: Consumer safety of enzyme detergents, *J. A. M. A.* 211: 2017, 1970.
- 14 Hendricks, M. H.: Measurement of enzyme laundry products dust levels and characteristics in consumer use, *J. Am. Oil Chem. Soc.* 47: 207, 1970.
- 15 Hammond, A. L.: Phosphate replacements: Problems with the washday miracle, *Science* 172: 361, 1971.
- 16 Bernstein, I. L.: Further studies on anaphylactic sensitization induced by *B. subtilis* enzyme, *Israel J. Med. Sci.* 6: 746, 1970.
- 17 Belin, L., Hoborn, J., Falsen, E., et al.: Enzyme sensitization in consumers of enzyme-containing washing powder, *Lancet* 2: 1153, 1970.
- 18 Falleroni, A. E., and Schwartz, D. P.: Immediate hypersensitivity to enzyme detergents, *Lancet* 1: 548, 1971.
- 19 Lowry, O. H., Rosebrough, N. J., Farr, A. L., et al.: Protein measurement with the Folin phenol reagent, *J. Biol. Chem.* 193: 265, 1951.
- 20 Shore, P. A., Burkhalter, A., and Cohn, V. H.: A method for the fluorometric assay of histamine in tissues, *J. Pharmacol. Exp. Ther.* 127: 182, 1959.
- 21 Salvaggio, J. E., Cavanaugh, J. J. A., Lowell, F. C., and Leskovitz, S.: A comparison of the immunologic responses of normal and atopic individuals to intranasally administered antigen, *J. ALLERGY* 35: 62, 1964.
- 22 Crane, R., and Burke, W. E.: The rhinorheometer: A new device for measuring nasal patency. Presented at the Twenty-third Annual Meeting of the ACEMB, Washington, D. C., Nov. 15-19, 1970.
- 23 Kory, R. C., Callahan, R., and Boren, H. G.: The Veterans Administration Army Co-operative Study of Pulmonary Function. I. Clinical spirometry in normal men, *Am. J. Med.* 30: 243, 1961.
- 24 Ogilvie, C. M., Forster, R. E., Blakemore, W. S., et al.: A standardized breath holding technique for the clinical measurement of the diffusing capacity of the lung for CO, *J. Clin. Invest.* 36: 1, 1957.
- 25 Nishimura, S., and Osawa, H.: Extracellular ribonuclease from *B. subtilis*. II. Its amino acid composition and structural comparison with bovine pancreatic ribonuclease, *Biochim. Biophys. Acta* 55: 421, 1962.

- 26 Lichtenstein, L. M., Norman, P. S., and Connell, J. T.: Comparison between skin-sensitizing antibody titers and leukocyte sensitivity measurements as an index of the severity of ragweed hay fever, *J. ALLERGY* 40: 160, 1967.
- 27 Levy, D. A., and Osler, A. G.: Studies on the mechanisms of hypersensitivity phenomena. XVI. In vitro assay of reaginic activity in human sera: Effect of therapeutic immunization on seasonal titer changes, *J. Immunol.* 99: 1068, 1967.
- 28 Dolovich, J., Debanne, M. T., and Wicher, V.: Antigenic compositions of enzyme-containing preparations of *Bacillus subtilis* origins, *J. ALLERGY* 47: 98, 1971. (Abst.)
- 29 Wicher, V. M., and Dolovich, J.: Interactions of *Bacillus subtilis* alkaline proteinases with α_2 -macroglobulin and α_1 -antitrypsin, *Int. Arch. Allergy Appl. Immunol.* 40: 779, 1971.
- 30 Berson, S., Yalow, R., Saito, T. O., et al.: Antibodies to "Alcalase" after industrial exposure, *N. Engl. J. Med.* 284: 688, 1971.
- 31 Bloch, K. J.: The anaphylactic antibodies in man, *Progr. Allergy* 10: 84, 1967.
- 32 Orange, R. P., and Austen, K. F.: Slow reacting substance of anaphylaxis, *Advances Immunol.* 10: 105, 1969.
- 33 Parish, W. E.: Short-term anaphylactic IgG antibodies in human sera, *Lancet* 2: 591, 1970.
- 34 Henningsen, S. J., and Zachariae, H.: Kinin formation and inactivation by Alcalase, a proteolytic enzyme, *Proc. Soc. Exp. Biol. Med.* 132: 459, 1969.
- 35 Audran, R., and Joyeaux, Y.: Inactivation du complement serique in vitro par des fractions de levane de *Bacillus subtilis*, *C. R. Acad. Sci. (Paris)* 250: 3909, 1960.
- 36 Kuo, J. F.: Comparison of the effects of *B. subtilis* protease type VIII (subtilopeptidase A) and insulin on isolated adipose cells, *J. Biol. Chem.* 243: 211, 1968.
- 37 Goldring, I., Ratner, I., and Greenburg, L.: Pulmonary hemorrhage in hamsters after exposure to proteolytic enzymes of *Bacillus subtilis*, *Science* 170: 73, 1970.
- 38 Dubos, R.: Toxic factors in enzymes used in laundry products, *Science* 173: 259, 1971.
- 39 Simon, A., and Pollak, A.: Glucose oxidase induced granulomatous lung disease, *Dis. Chest* 56: 98, 1969.
- 40 Morihara, K., and Tsuzuki, H.: Comparison of the specificities of various serine proteinases from microorganisms, *Arch. Biochem. Biophys.* 129: 620, 1969.
- 41 Imanishi, A., Monotani, Y., and Isemura, T.: The interaction of detergents with proteins, *J. Biochem.* 57: 417, 1965.
- 42 MacLaren, K. R., and Aladjem, F.: Allergy to chymotrypsin, *J. ALLERGY* 28: 89, 1957.
- 43 Rose, K. D.: Anaphylactic reaction to aqueous chymotrypsin injection. Development in a previously nonsensitive patient, *J. A. M. A.* 173: 196, 1960.
- 44 Liebowitz, D., and Ritter, H., Jr.: Anaphylactic reaction to chymotrypsin, *J. A. M. A.* 172: 159, 1960.
- 45 Luft, S.: Reactions to parenteral enzyme therapy, *J. Am. Podiatry Assoc.* 50: 209, 1960.
- 46 Howell, I. L.: Anaphylactic reaction to chymotrypsin, *J. A. M. A.* 175: 322, 1961.
- 47 Howe, C., Erlanger, B. F., Beiser, S. M., et al.: Hypersensitivity to purified trypsin and chymotrypsin, *N. Engl. J. Med.* 265: 332, 1961.
- 48 Gherini, A. J., and Hovde, D.: Anaphylactic reactions to chymotrypsin. A report of two cases, *Calif. Med.* 94: 379, 1961.
- 49 Sherline, D. M., and Blackwell, W. J.: Anaphylactic reaction to chymotrypsin. An obstetrical case report, *J. A. M. A.* 181: 1079, 1962.
- 50 Ferrara, A., Finke, S., Grieco, M. H., Connell, J. T., and Sherman, W. B.: Anaphylactic reactions to aqueous chymotrypsin, *J. ALLERGY* 34: 460, 1963.
- 51 Crip, L. H., and Beam, L. R.: Allergy to trypsin, *J. ALLERGY* 35: 425, 1964.
- 52 Editorial: Organic dusts and allergic lung diseases, *Lancet* 1: 1195, 1969.
- 53 Editorial: Sensitization to the enzymes in detergents, *J. ALLERGY* 47: 53, 1971.
- 54 Editorial: Pollution through cleanliness, *N. Engl. J. Med.* 284: 723, 1971.
- 55 Brodeur, P.: A reporter at large: The enigmatic enzyme, *The New Yorker*, January 16, 1971, p. 42.