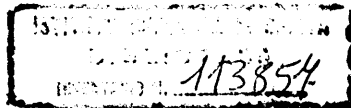


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Respiratory Symptoms and Skin Reactivity in Workers Exposed to Proteolytic Enzymes in the Detergent Industry^{1,2}

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SUMMARY

A survey of 98 workers in the detergent industry exposed periodically to high concentrations of proteolytic enzymes indicated that symptoms suggestive of asthma developed in 50 per cent on exposure either within one-half hour, after four or five hours, or at night. Immediate (prick) and five-hour (intradermal) cutaneous reactions to the enzymes were not significantly more frequent in the symptomatic (72 per cent and 44 per cent, respectively) than in the asymptomatic subjects (56 per cent and 24 per cent). By contrast, there was a highly significant association between skin reactions to the enzyme and to three common inhalant allergens. With the exception of a history of food and drug allergy, a personal or family history of atopic manifestations did not influence the likelihood of development of symptoms, nor did smoking habit or current, clinical evidence of bronchitis. The findings suggest that symptomatic bronchial reactions in persons exposed to a potent sensitizing and chemically active material are determined by other factors in addition to allergy, at least as indicated by skin reactivity. Tests of ventilatory and diffusing capacity showed no evidence of permanent lung damage in sensitized or unsensitized subjects, whether symptomatic or not.

Introduction

In 1968, a patient working in the detergent industry presented in this unit complaining of asthma, and an occupational basis was suspected on the basis of the history. An extract of a proteolytic enzyme used in the detergent powders produced skin reactions, and inhala-

tion under controlled conditions produced a decrease in ventilatory capacity; negative skin and inhalation tests were obtained with proteolytic enzymes from other sources.

Six months later, after the initial report of Flindt (1), complaints of respiratory symptoms by employees handling proteolytic enzymes in the manufacture of detergent products led to a request for more comprehensive investigation. The first stage of this study, undertaken after 18 months of enzyme detergent production, was a survey to determine the prevalence and nature of symptoms among employees of one factory. In addition, details of previous respiratory and allergic disorders were sought and pulmonary function was examined for evidence of permanent disability. The present paper is con-

(Received in revised form February 9, 1971)

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cerned with the results of immediate, practical importance derived from this survey. As the opportunity to study a relatively large number of subjects hyperreactive to a single inhalant is uncommon, the clinical features are presented in some detail, especially as they are significant in regard to the possible mechanisms involved.

Materials and Methods

The industrial process: Proteolytic enzymes have been used increasingly in the detergent industry in prewashing or soaking agents. "Alcalase," a commercial enzyme-containing preparation manufactured from the submerged fermentation of *Bacillus subtilis*, contains approximately 10 per cent to 15 per cent of organic matter, mostly protein with some carbohydrate, 25 per cent sodium sulfate, and 65 per cent sodium chloride. The active enzyme, an alkali-stable endopeptidase with a relatively broad spectrum of proteolytic activity, constitutes 6 per cent (weight/weight) of the commercial material.

Initially, Alcalase, a fine dusty powder, was diluted by a factor of four with sodium sulfate, and 2 per cent mineral oil was added to reduce dust formation. The resulting complex was further diluted by a factor of 25 or 50 with standard detergent powders to give the final products, which were a pure soaking agent containing 1 per cent commercial Alcalase or a detergent powder containing 0.5 per cent Alcalase. These mixtures were stored in tote bins and later fed into hoppers supplying automatic packet-filling machines. Damaged packets were sent to a reclaiming area, where they were "cut up," and their contents were transferred to filling hoppers. Initially, enzyme-containing detergents were produced in "runs" lasting two days to five days every few weeks.

During the first 18 months of this process, there were considerable problems in dust control, particularly in the enzyme concentrate area, where employees would transfer the crude enzyme by hand scoop without masks or effective ventilation. Other dusty zones at this time were the feeding points for the hoppers, the packeting line (when accidents occurred), and the "cutting-up" area.

After 18 months, various dust control measures, together with regular measurements of enzyme activity in airborne dust, were introduced,⁴ when average values for enzyme activity

in the air were of the order of 5 glycine units (1.17 glycine units equals 1 μg of Alcalase) per m^3 , with values as large as 150 glycine unit per m^3 when a major spillage occurred.⁵

Personnel: At the time of the survey, 175 employees were involved in the production of enzyme-containing detergent powders. Of these, 98, comprising all but 10 of those working full-time in the preparation of detergent, took part in the survey. Most of the remaining 77 worked in peripheral areas such as the store, entered the main production area infrequently, and had negligible or slight exposure; the others worked as maintenance or supervisory staff. Symptoms of the lower respiratory tract developed on exposure in at least 3 of the 10 subjects who were not studied but who had moderately consistent exposure to enzymes. Other subjects were known to have left the industry since the introduction of enzymes because of respiratory symptoms.

The working day was divided into three shifts: morning, 7 A.M. to 3 P.M.; evening, 3 P.M. to 11 P.M.; night, 11 P.M. to 7 A.M. Most employees always worked the same shift.

Reference is made subsequently to subjects having "mild," "moderate," or "heavy" exposure during the 18 months of enzyme usage before the study, and particularly to subjects reacting with symptoms either on "mild" or on "moderate" exposure. This classification was inevitably arbitrary because no dust measurements were available during the relevant period. The grouping was primarily based on the subject's own assessment, his particular job, and a knowledge of its likely risks based on subsequent dust measurements. It was obviously subject to error, notably in that symptomatic subjects were likely to overestimate their exposure and asymptomatic ones to underestimate it. Its sole purpose, however, was to attempt to identify the more susceptible or "hypersensitive" subjects. In general, "heavy" exposures were those associated with accidental spillages.

Ninety-eight employees, 67 men and 31 women, of mean ages 36 years and 32 years, respectively, took part in the study. Because the small excess of symptomatic subjects among the men could readily be explained on the basis of heavier exposure and smoking habits, and because no other differences were apparent on analysis, the sexes were not considered separately.

⁵ Subsequent improvements have produced lower average concentrations of 0 glycine unit to 1 glycine unit per m^3 with occasional values of 1 glycine unit to 2 glycine units per m^3 in the hopper filling area.

⁴ Simson, R., and Simpson, G. R.: Submitted for publication.

ly. The following investigations were carried out.

Observed cough: Before the history was taken or examination performed, each subject was asked to take a big breath and to cough forcibly. This cough was adjudged by ear to be either "loose" (productive) or "dry" (unproductive). This test provides reliable objective evidence of sputum production (2).⁶

Questionnaire: Respiratory symptoms, past and family history of chest diseases, and smoking habits were recorded for each worker using the British Medical Research Council questionnaire on respiratory symptoms (1960) (3) with some additions concerning atopic manifestations. These questions were related to the period before the use of enzymes in the plant. Employees were then asked the deliberately vague question "Do the enzymes affect you in any way?" If no respiratory symptoms were volunteered, specific inquiry was made regarding nasal symptoms, cough, sputum, wheeze, chest tightness, and shortness of breath during or after exposure.

The temporal relationship of respiratory symptoms to exposure and to the time of day was particularly noted; previous experience had directed attention to the significance of these considerations (4, 5).

Pulmonary function tests: The one-second forced expiratory volume (FEV_1) and forced vital capacity (FVC) were measured in all subjects with a McDermott direct reading dry spirometer. The largest of three consistent (within 10 per cent) estimates was used in the analysis. The ratio, FEV_1/FVC , was calculated and expressed as a percentage. The results were compared with the expected values for vital capacity (VC) from the prediction formulas of Goldman and Becklake (6) on the basis of age, standing height, and sex. The expected FEV_1 was calculated by assuming FEV_1/FVC to be 80 per cent in subjects less than 40 years old and 75 per cent in those older than 40 years, and relating this to the predicted VC.

A standard exercise step test was performed by most of the subjects who admitted to any symptoms. A work level of 350 kg m per min for five minutes was used (approximately ten flights of stairs for a 70 kg man), and the standardized ventilation, adjusted to a work load of 300 kg m per min (SV_{300}) was calculated (7).

The steady state diffusing capacity for carbon monoxide (DL_{CO}) was measured at rest using end-tidal sampling, and the results were compared with expected normal values (8).

⁶ Hall, G. J. L., and Gandevia, B.: Submitted for publication.

Bronchial reactivity was assessed as the percentage change in FEV_1 after inhalation of an aerosol of histamine phosphate. The procedure and results will be described in detail elsewhere.

Skin sensitivity tests: Skin tests could not be performed for two months after the initial survey. Eighteen of the 98 original subjects were not tested because they were on night shift or had left the industry for nonmedical reasons; although most have since been tested, the time lapse is considered too great to allow their inclusion in the present analysis. Also tested was a group of 75 employees not directly involved in the production of enzyme-containing detergents, and only occasionally and transiently exposed to them; most were among the 77 employees mentioned previously as working in peripheral areas. Prick tests were performed using two extracts of commercial Alcalase in a concentration of 1 mg per ml, and also extracts of house dust, *Aspergillus*, and spring grass pollens (Biotech, C strength). Results were read at 20 minutes and recorded as nonreactive when no palpable wheal was present, reactive (1 plus) when a wheal up to 4 mm diameter was present, or strongly reactive (2 plus) when the reaction was larger than this. Intradermal tests were performed using 0.01 ml to 0.02 ml of the Alcalase extract in a concentration of 0.05 mg per ml. Results were read at five hours and recorded as the mean diameter of induration in millimeters. The distribution of reaction size in the 80 subjects tested (figure 1) showed a marked change at 8 mm to 9 mm, such that reactions of 8.5 mm or less were classified as nonreactive. Support is given to this interpretation by the highly significant association of reactions greater than 8.5 mm with immediate reactions ($\chi^2 = 33.30$; $P < 0.0005$).

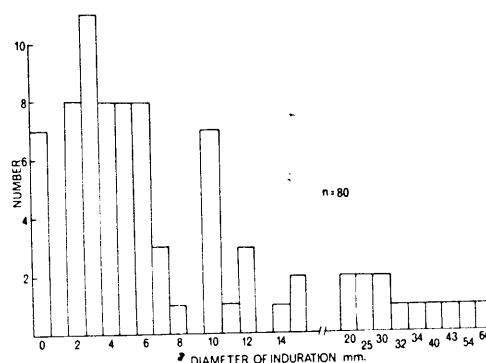


Fig. 1. Delayed skin reactions to the intradermal injection of 0.01 ml to 0.02 ml of Alcalase extract in a concentration of 0.05 mg per ml measured at five hours in 80 subjects.

One of the Alcalase extracts was prepared by the Department of Clinical Immunology, Institute of Diseases of the Chest, Brompton Hospital, London, and the other was prepared in this laboratory by the method of Pepys and associates (9). The reactions to the two extracts were almost identical and only the results of the reactions to the preparation made in this laboratory are presented.

Chest radiographs: Posteroanterior chest radiographs were obtained in all subjects at or about the time of the survey and were read independently by three observers.

Results

Upper respiratory tract symptoms: Sneezing, nasal obstruction, and rhinorrhea occurred in 60 per cent of the subjects, usually within one-half hour of exposure, and continued for approximately two hours after cessation. Nasal symptoms on exposure to other detergent preparations occurred but were less common and less severe than with the enzyme-containing products. Conjunctival irritation and lachrymation were occasionally noted in association with nasal symptoms. In 15 per cent of the affected subjects, nasal symptoms were accentuated on re-exposure after a few days away from work. Current nasal symptoms on exposure to enzyme

(table 1) were not associated with a past history of nasal symptoms ($\chi^2 = 2.40$; $P > 0.1$) but were significantly related to lower respiratory tract symptoms on enzyme exposure ($\chi^2 = 5.79$; $P < 0.01$).

Lower respiratory tract symptoms: Lower respiratory tract symptoms occurred in 49 subjects (50 per cent). The most common symptom, "tightness in the chest," occurred in 37 of these subjects; those more severely affected complained of concomitant "shortness of breath." Nonproductive cough and frank wheezing were less frequent. The cough occurred when the tightness in the chest was maximal, but it was also often associated with smoking a cigarette; many heavy smokers were unable to smoke, and occasionally this was the predominant symptom. Sputum production after exposure was a feature in 7 subjects who said that they produced small volumes of brown sputum, not of the usual mucoid appearance, six to eight hours after exposure.

Only one subject denying respiratory symptoms on exposure to enzyme reported a recent reduction in exercise tolerance whereas 6 of those with symptoms on exposure (on minimal exposure in 5 subjects) did so.

The time of onset of lower respiratory tract symptoms was described as immediate (within 0.5 hour), delayed (either four hours to five hours, or nocturnal, between 11 P.M. and 3 A.M.). In 13 of the 49 affected subjects, the onset of lower respiratory symptoms was within 0.5 hour of exposure to the enzyme preparation. This exposure was of moderate intensity; only one subject, the only one to experience the more common delayed symptoms, reacted to minimal exposure. Symptoms rarely lasted longer than 24 hours and never longer than two days (table 2). Immediate reactions of this kind did occur to a first exposure, particularly if the exposure was exceptionally heavy, and in the presence of non-reactive skin tests.

In the other 36 affected subjects, the onset of symptoms was delayed for four hours to five hours after exposure in 28 subjects, and until night-time, between 11 P.M. and 3 A.M., in the other 8. In 17 of the 28 subjects, nocturnal symptoms occurred in addition to a

TABLE 1
RELATIONSHIP BETWEEN NASAL
SYMPTOMS ON EXPOSURE TO ENZYMES
AND PAST NASAL SYMPTOMATOLOGY
LOWER RESPIRATORY TRACT SYMPTOMS
ON EXPOSURE AND SKIN SENSITIVITY
REACTION TO ENZYMES

	Nasal Symptoms on Exposure	
	Present	Absent
No. of subjects	62	36
Past history of catarrh	18	5
Lower respiratory tract symptoms on exposure	38	11
Skin reactions		
No. tested	55	25
Immediate (prick test)		
Positive	39	12
Negative	16	13
Delayed (5 hours) (intradermal test)		
Positive	20	7
Negative	35	18

TABLE 2
THE RELATIONSHIP BETWEEN THE TIME
OF ONSET OF SYMPTOMS, THEIR
DURATION, AND EXPOSURE REQUIRED

	Onset of Symptoms	
	Immediate	Delayed
No. of subjects	13	36
Duration of acute symptoms		
Less than two hours	7	0
Two to 24 hours	4	16
One to three days	2	13
More than three days	0	6
Exposure necessary to produce symptoms		
Minimal	1	20
More than minimal	12	16
Recent dyspnea	2	4

four- to five-hour reaction after an asymptomatic, or relatively asymptomatic, phase during the evening. Subjects with delayed symptomatic reactions had usually been exposed to enzymes for several months before symptoms of this nature developed, although during this latent period accidental heavy exposures produced symptoms immediately after exposure in 3 subjects. Significantly more of those with a delayed onset reacted to mini-

mal exposure (20 subjects or 56 per cent) than to moderate exposure (16 subjects or 44 per cent) ($\chi^2 = 8.93$; $P < 0.005$) (table 2), in contrast to the subjects with an immediate onset of symptoms. Further, their symptoms were significantly more persistent, lasting for longer than 24 hours in 57 per cent of subjects and for longer than three days in 16 per cent ($\chi^2 = 23.02$; $df = 3$; $P < 0.0005$). If symptoms in the group with nocturnal onset or exacerbation persisted for longer than 24 hours, symptoms recurred during the second and occasionally the third night, so that these subjects might have symptoms on Saturday, and sometimes Sunday, nights in the absence of exposure and symptoms during those days. If exposure occurred during a night shift, symptoms were either immediate or delayed for four to five hours; an exacerbation of symptoms was not observed during the day at a time corresponding to the 11 P.M. to 3 A.M. period of those subjects working the day shift. In fact, one subject, who initially worked on the morning shift, transferred to the permanent night staff because his symptoms at night had prevented him from sleeping; on the night shift

TABLE 3
RELATIONSHIP BETWEEN PRESENCE AND TIME OF ONSET OF LOWER
RESPIRATORY TRACT SYMPTOMS AND PRESENCE OF A PRODUCTIVE
COUGH, PAST AND FAMILY HISTORY OF CHEST DISEASE, AND ATOPY

	Symptomatic			Asymptomatic
	Immediate Onset	Delayed Onset	Total	
No. of subjects	13	36	49	49
Loose cough by test	4	13	17	19
History of cough and sputum	7	19	26	21
Prior wheeze	4	6	10	4
Chronic or winter catarrh	4	10	14	8
Seasonal catarrh	0	0	0	1
Bronchitis in the past 3 years	2	5	7	9
Previous illnesses				
Asthma	1	1	2	2
Bronchitis	4	7	11	14
"Chesty" childhood	0	5	5	4
Eczema	1	2	3	2
Hives	2	1	3	5
Drugs or food allergy	3	8	11	1
Family history				
Asthma	3	7	10	9
Hay fever	2	2	4	4
Other allergy	1	0	1	0

his nocturnal symptoms were not sufficiently troublesome to prevent him from working, and at no time did symptoms develop during the day.

Headache: Headache on exposure was volunteered by 10 per cent of subjects, usually three to four hours after exposure, but occasionally within 0.5 hour. It was severe, throbbing, and localized to the frontal and temporal regions, lasting for about four hours or until relieved by aspirin. Headache occurred independently of respiratory symptoms.

Relationship of Occupational Symptomatology to Past and Family Histories, Smoking Habits, Skin Tests, and Pulmonary Function

The results of the observed loose cough and data obtained from the questionnaire are set out in table 3 in relation to the symptomatic and asymptomatic groups. Except for a significantly greater prevalence of a past history of drug or food allergy ($\chi^2 = 9.5$; $P < 0.005$), the various features listed occurred with similar frequency in both groups. In particular, the other possible manifestations of atopy, such as seasonal catarrh, asthma, eczema, or family histories of asthma, hay fever, and other allergies, were not more frequent in the symptomatic group. This group also showed no excess of past or present manifestations of bronchitis, including the relatively objective test of an observed loose cough. The distinction between those with immediate and those with delayed onset of symptoms has been preserved in the table,

because (with one exception) they were mutually exclusive and because of the theoretic implications with regard to possible mechanisms. Comparison between these subgroups, however, also failed to reveal important differences, although all 5 subjects giving a history of "chestiness" in childhood were among those with delayed symptomatic reactions.

The smoking habits of subjects in the same three categories are shown in table 4. Fewer asymptomatic subjects currently smoked, and the total cigarette consumption was significantly less in this group than in the symptomatic groups combined ($t = 2.39$; $P < 0.02$) or in the subgroup with delayed symptomatic reactions alone ($t = 2.37$; $P < 0.025$). Daily cigarette consumption of current smokers was similar.

Skin sensitivity reactions: Of the 80 subjects tested, 51 (64 per cent) showed immediate cutaneous reactions (table 5); 26 of these showed no delayed reactions. Delayed skin reactions (> 8.5 mm) were recorded in 27 (34 per cent); only 2 of these subjects had no immediate reactions. Immediate type sensitivity thus appeared to develop earlier or more readily.

The results of skin sensitivity tests in subjects with upper respiratory tract symptoms on exposure to enzyme are shown in table 1. Immediate reactions were observed in 71 per cent of 62 such subjects, and delayed reactions, in 36 per cent. For those without nasal symptoms, the corresponding figures were 48 per cent and 28 per cent. The association of immediate reactions with nasal symptoms was significant ($\chi^2 = 3.90$; $P < 0.05$) and re-

TABLE 4
SMOKING HABITS IN THE MAJOR SYMPTOM GROUPS

	Immediate Onset	Delayed Onset	Asymptomatic
No. of subjects	13	36	49
Nonsmokers	3	3	11
Ex-smokers	3	3	4
Current smokers	7	30	34
Mean ($\pm$ SD) daily cigarette consumption in current smokers	22.9 $\pm$ 5.7	18.8 $\pm$ 7.6	19.8 $\pm$ 7.0
Total cigarette consumption for whole group, pack-years	15.8 $\pm$ 16.4	18.3 $\pm$ 16.8	10.5 $\pm$ 12.3

TABLE 5
SKIN REACTIONS TO ALCALASE IN THE MAJOR SYMPTOM GROUPS

	Symptomatic			Asymptomatic
	Immediate Onset	Delayed Onset	Total	
No. of subjects tested	11	28	39	41
Immediate reaction to prick test				
Positive	7	21	28	23
Negative	4	7	11	18
Delayed reaction to intradermal test (at five hours)				
Positive	5	12	17	10
Negative	6	16	22	31

mained so after exclusion of the 38 subjects who also had lower respiratory tract symptoms ($\chi^2 = 4.39$; $P < 0.05$).

By contrast, the relationship between lower respiratory symptoms and skin sensitivity was poorly defined (table 5). No significant relationship was demonstrable between the overall presence or absence of symptoms on exposure and the presence of immediate ($\chi^2 = 2.13$; $P > 0.1$) or delayed ($\chi^2 = 3.29$; $P > 0.05$) skin reactions compared with the asymptomatic group. When the asymptomatic subjects and those with immediate or with delayed onset of symptoms were considered as three separate groups, there was again no significant evidence of a relationship in respect to either immediate ($\chi^2 = 1.68$; $df = 2$; $P > 0.4$) or delayed ($\chi^2 = 3.32$; $df = 2$; $P > 0.1$) skin reactions.

This unexpected finding prompted the analysis of skin reactions in relation to the degree of exposure required to produce symptoms on the hypothesis that evidence of allergy might be more readily demonstrable in subjects in whom lower respiratory symptoms developed on minimal, as distinct from moderate or heavy, exposure. Of the 39 symptomatic subjects tested, symptoms developed in 19 on minimal exposure whereas symptoms developed in 20 only on heavier exposures. Immediate skin reactions were found in 15 of the former and 13 of the latter whereas delayed reactions developed in 8 and 9, respectively. These figures did not indicate any significant difference in terms of skin sensitivity between these two groups or

between either of them and the asymptomatic group.

The relationship of immediate reactions on prick testing to Alcalase and to three common allergens (house dust, *Aspergillus*, and spring grass pollens) in 155 subjects is shown in table 6. They comprised the 80 subjects tested in the present series and 75 subjects occasionally and mildly exposed to the enzyme. The potency of the enzyme as a sensitizing agent was illustrated by the similarity in the proportion of reactors in both groups (64 per cent and 52 per cent, respectively). Of the 155 subjects, 62 (40 per cent) reacted to at least one of the common allergens, a prevalence similar to that found by other investigators in or out of the industry (10, 11).

The association between reactions to

TABLE 6
RELATIONSHIP BETWEEN IMMEDIATE REACTIONS TO ALCALASE AND IMMEDIATE REACTIONS TO THREE COMMON ALLERGENS

Reactions to Three Common Allergens*	Reactions to Alcalase	
	Positive	Negative
Three positive	7	0
Two positive	12	3
One positive	29	11
All three negative	42	51
Total	90	65

*The allergens were house dust, *Aspergillus*, spring grass pollens.

$\chi^2 = 11.80$; $df = 3$; $P < 0.005$.

TABLE 7
MEAN ($\pm$ SD) VALUES FOR TESTS OF VENTILATORY CAPACITY,
DIFFUSING CAPACITY, AND SV_{300} IN THE MAJOR SYMPTOM GROUPS

	Immediate Symptoms Only	Delayed Symptoms	Asymptomatic
No. of subjects	12	37	49
Mean age, years	34.8	36.5	34.1
FEV ₁ , % predicted	97.6 $\pm$ 11.7	99.0 $\pm$ 15.6	98.4 $\pm$ 13.8
FVC, % predicted	95.7 $\pm$ 8.8	100.0 $\pm$ 13.3	95.2 $\pm$ 9.6
FEV/FVC, %	77.8 $\pm$ 8.7	76.7 $\pm$ 7.8	78.9 $\pm$ 7.5
Diffusing capacity, % predicted*	95.4 $\pm$ 27.4 (7) [†]	94.5 $\pm$ 23.3 (19)	91.8 $\pm$ 8.9 (4)
SV ₃₀₀ , liter/min	36.8 $\pm$ 7.6 (8)	34.1 $\pm$ 7.7 (31)	34.8 $\pm$ 6.8 (15)

* Values obtained with tidal volumes less than 500 ml were not analyzed.

[†] Figures in parentheses indicate number of subjects when less than group total.

Alcalase and other allergens was strong ($\chi^2 = 17.8$; $P < 0.0005$). The potency of the enzyme as a sensitizing agent was again reflected in the finding that 45 per cent of the 93 subjects with no reactions to other allergens showed reactions to Alcalase.

Pulmonary function: Preliminary analysis revealed the expected trends in ventilatory capacity in relation to smoking habits but no significant differences between smokers, nonsmokers, and ex-smokers. Nonsmokers and ex-smokers were grouped together in the subsequent analysis. The results of the pulmonary function tests performed in the symptomatic and asymptomatic groups are shown in table 7. No significant differences were present with regard to FEV₁, FVC, and DLCO (expressed as a percentage of predicted normal values) nor in FEV₁/FVC or SV₃₀₀.

In an attempt to check further on the presence or absence of long term effects of enzyme exposure, all subjects, regardless of symptoms, were classified as having "mild," "moderate," or "heavy" exposure during the total period of 18 months; reanalysis in these terms also failed to reveal any significant functional differences. Comparison of the symptomatic, cutaneous reactors (who might be considered most susceptible) with asymptomatic subjects produced no evidence of impaired lung function.

Chest radiographs: No relevant abnormalities were observed in the lung fields in any of the chest radiographs.

Discussion

The causal relationship between enzyme inhalation and respiratory symptoms became evident to the employees after relatively brief experience. This conclusion was facilitated by the high proportion affected, by the episodic nature of enzyme "runs," and by the rapid onset of symptoms in some cases. Subjects with nocturnal symptoms only, or with nocturnal exacerbations, sometimes posed a diagnostic problem for their physicians, especially if the nocturnal symptoms continued after exposure had ceased. This pattern of reaction, attributed to inhibition of endogenous mechanisms tending to maintain normal ventilatory function during the day (4), has been described in relation to Western red cedar (*Thuja plicata*) (5) and other occupational inhalants (12) and is recognized after house dust challenge and hyposensitization injections. The validity of description in terms of immediate, delayed,⁷ and nocturnal reactions has been confirmed in 29 subjects by serial estimations of ventilatory capacity after bronchial challenge with an extract of Alcalase (13); these studies also confirmed the clinical impression that lower respiratory symptoms were attributable to the development of airway obstruction or asthma.

The hypothesis that respiratory symptoms

⁷ The word "delayed" is used in its usual sense with no immunologic connotation.

on exposure to proteolytic enzymes have an allergic basis (9) requires critical examination. Nasal symptoms, the most common expression of an abnormal reaction, were of rapid onset and significantly associated with immediate prick test reactions in 71 per cent of those affected, features suggesting immediate or type 1 (14, 15) hypersensitivity; however, immediate skin reactions were not significantly more common in the small group of 11 subjects with immediate lower respiratory tract symptoms than in 41 asymptomatic subjects. Similarly, the frequency of late (four hours to five hours) skin reactions was not significantly greater in those with delayed symptomatic reactions than in the asymptomatic group, a finding that does not support a determining role for type 3 hypersensitivity (14, 15). Although larger numbers might produce associations of statistical significance, the fact remains that approximately 30 per cent of symptomatic subjects showed no skin reactions even though the enzyme produced skin reactivity in more than one-half the asymptomatic subjects.

There are three possible explanations for these findings. The first possibility, that symptomatic subjects as a group tended to report nonexistent or coincidental symptoms, can be excluded, if only because of the consistency of the histories and the characteristic temporal patterns. Newhouse and associates (12) appeared to take the view that, among 117 symptomatic subjects in their survey, the 64 per cent who had nonreactive skin tests were reporting respiratory symptoms due to non-industrial causes. This explanation is untenable in the present series, in which symptoms were encountered regardless of the season and in which there was no notable tendency to chronic bronchitis and no remarkable epidemic of respiratory infection. Proof that bronchial reactions of appropriate temporal relationships occur in symptomatic but nonreactive subjects has been provided by bronchial challenge tests using small aerosol doses of Alcalase extract (0.1 mg per ml) (13).

The second possibility is that these subjects were hypersensitive but failed to show evidence of hypersensitivity in the skin. This is

conceivable but surprising in view of the large over-all prevalence of skin reactions.

The third possibility is that the bronchial reactions were due to a direct irritant effect of the enzyme, perhaps related to its biologic activity. Wilhelm (16) and his co-workers have differentiated six patterns of inflammatory response to chemical and thermal stimuli primarily on the basis of the temporal relationship of the response to the initiating stimulus. Types 1 and 3 of Wilhelm show the same time relationships as the immediate and late hypersensitivity reactions (types 1 and 3 of Gell and Coombs) (14). Using change in FEV_1 as the index of response instead of an index of vascular permeability, the same six temporal patterns, particularly the immediate and late patterns, were demonstrable after bronchial challenge with Alcalase in 29 symptomatic subjects of the present series. Further evidence to suggest a direct inflammatory effect was provided by the development of diffuse fine rales in 11 of these 29 subjects within hours of bronchial challenge with a dose of Alcalase within the range likely to be encountered during a day's work under the old unsatisfactory working conditions or after accidental spillages (17); this reaction was observed regardless of skin reactivity, although it was perhaps more likely to occur in response to very small concentrations (0.01 mg per ml) in those with skin reactions. In fact, it was the occurrence of these reactions that obliged the present writers to discontinue further studies of this type. Finally, papain, also an endopeptidase, has been shown to produce pulmonary reactions in several animal species, characterized "clinically" by cough (18) and histologically by hemorrhagic inflammation ultimately leading to emphysema (19, 20).

Although a conclusion as to the relative significance of hypersensitivity and direct irritation in producing symptoms is not possible from the available evidence, the practical implications of the present investigation are clear. First, all the subjects affected by enzyme exposure will not necessarily be identified within the group showing immediate reactions to the prick tests; second, the real

possibility that permanent damage can occur, at least after exposures that are excessive by current standards, demands a more comprehensive medical surveillance than one based primarily on the development of cutaneous reactions. These views contrast with the current recommendations of a United Kingdom industry committee and the suggestions of Newhouse and co-workers (10).

In the present investigation, the likelihood of symptoms developing on exposure to the enzyme was not related to past respiratory history, the presence of a productive cough (as judged by the history or the character of a requested cough), or to current smoking habits. More surprisingly, various indices of the atopic state in the past and family histories showed no greater prevalence in the symptomatic than the asymptomatic group, with the exception of a history of food or drug allergy. There was a strong association between the presence of a cutaneous reaction to Alcalase and cutaneous reactions to three common allergens, although approximately 25 per cent of the 155 subjects showed reactions to Alcalase and no reactions to the other allergens. It follows, from this and earlier observations on the relationship between symptoms and skin reactions, that atopy, if defined in terms of liability to reactive skin responses, influences the likelihood of a cutaneous skin reaction to Alcalase developing considerably more than it relates to the likelihood of respiratory symptoms developing. It should be stressed that the environmental dust concentrations to which the subjects were exposed were very high by current standards; the degree of atopy of the population might assume relatively greater importance when the exposure is less. In any case, factors other than skin test reactivity appear to contribute to the development of symptoms, even within the cutaneous reactors or atopic group; evidence that bronchial reactivity to histamine plays some part will be presented in a later paper.

Pulmonary function tests, including estimations of ventilatory and diffusing capacity, revealed no statistical evidence of persisting bronchopulmonary disorder in the subjects. The series is inevitably biased, and this find-

ing cannot be taken to imply the absence of harmful effects on long term exposure. In fact, there were 4 patients with respiratory symptoms that apparently persisted months after cessation of exposure, 2 of whom showed progressive decrease in pulmonary elastic recoil pressures, a change consistent with the development of emphysema (17).⁸ Although these observations are inconclusive, they emphasize the need for elaborate and comprehensive medical surveillance that should take the form of a prospective epidemiologic study and include subjects who do not react to the skin test (the ones perhaps more likely to tolerate heavy exposures without complaint) as well as follow-up studies in those in whom symptoms develop. It should initially include serial assessments of the mechanical properties of the lungs in selected personnel as a sensitive means of assessing increasing airway resistance or loss of elastic recoil. Obstructive syndromes appear to be the more likely possible hazards of long term exposure; so far clinical, radiologic, or functional evidence of alveolitis or interstitial disease were not found either in this survey, in clinical examinations of workers during enzyme "runs," or in response to deliberate aerosol challenge.

Acknowledgment

The writers thank Dr. A. Bell and Dr. R. Simson of the Division of Occupational Health, Department of Health, New South Wales, for making this study possible; the employees, medical staff, and management of the factory concerned for their cooperation; and the technical and secretarial staff for their assistance.

RESUMEN

Síntomas respiratorios y tendencia reactiva cutánea en trabajadores expuestos a enzimas proteolíticas en la industria de detergentes

Una investigación en 98 trabajadores en la industria de detergentes expuestos periódicamente a altas concentraciones de enzimas proteolíticas indicó que 50 por ciento desarrollan síntomas

⁸ Gandevia, B., and Mitchell, C.: The dangers of proteolytic enzymes to workers. *Med. J. Aust.*, 1971, 1, 1032.

que sugieren asma en exposición, bien sea a la media hora, después de 4 o 5 horas, o por la noche. Reacciones positivas inmediatas (Picadura) y de 5 horas (intradermal) no fueron significativamente más frecuentes en los individuos sintomáticos (72 por ciento y 44 por ciento respectivamente) que en los individuos asintomáticos (56 por ciento y 24 por ciento). En contraste, hubo una asociación significativamente alta entre las tendencias reactivas cutáneas positivas con enzimas y con 3 alérgenos inhalantes comunes. Exceptuando una historia de alergia a comidas o a drogas, la historia personal o familiar de manifestaciones atópicas no influyó la probabilidad del desarrollo de síntomas, ni tampoco el hábito de fumar o la evidencia clínica corriente de bronquitis. Los hallazgos sugieren que las reacciones sintomáticas bronquiales en una población expuesta a un sensibilizante potente y a un material químico activo pueden ser determinadas por medio de otros factores además de la alergia, por lo menos como lo indica la tendencia reactiva cutánea. No se encontró ninguna evidencia de daño pulmonar permanente, tal como lo indican las pruebas de la capacidad ventilatoria y difusoria en individuos sensibilizados o no sensibilizados, sintomáticos o no.

RESUME

Des symptomes respiratoires et de la réactivité de la peau parmi les ouvriers exposés aux enzymes protéolytiques dans l'industrie des produits détersifs

Un aperçu de 98 ouvriers dans l'industrie des produits détersifs, qui avaient été exposés périodiquement à des concentrations élevées des enzymes protéolytiques, indique que 50 pourcent de ces ouvriers développait des symptomes qui suggéraient l'asthme, ou bien au moment de l'exposition, ou dans une demi-heure, après quatre ou cinq heures, ou encore pendant la nuit. Les réactions positives immédiates (piqûres) et de cinq heures (intradermiques) n'étaient pas tellement plus fréquentes chez les sujets symptomatiques (respectivement 72 pourcent et 44 pourcent) que chez les sujets symptomatiques (56 pourcent et 24 pourcent). Par contre, il y avait une association extrêmement significative entre les réactions positives de la peau au détersif et aux trois allergènes inhalants communs. A l'exception d'une histoire d'allergie à certains aliments ou aux produits pharmaceutiques, ni une histoire personnelle ou

de famille de manifestations atopiques, ni l'habitude de fumer ou l'évidence clinique de bronchite avaient de l'influence sur la probabilité du développement des symptomes. On en conclut que les réactions symptomatiques bronchiques chez une population exposée à une matière puissante sensibilisante et active chimiquement peuvent être déterminées par d'autres facteurs ainsi que l'allergie, au moins comme c'est indiqué par la réactivité de la peau. L'évidence des tests de la capacité ventilatoire et de diffusion n'a pas démontré du dommage permanent des poumons parmi les sujets sensibilisés ou non-sensibilisés qu'ils soient symptomatiques ou non.

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