

MEDICAL PROBLEMS IN THE DETERGENT INDUSTRY CAUSED BY PROTEOLYTIC ENZYMES FROM BACILLUS SUBTILIS

By

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Proteolytic enzymes from *Bacillus subtilis* (subtilisins) have been used as ingredient in washing powders since 1963, when a "biological detergent" was introduced in Holland (20). Two types of subtilisins have been found—subtilisin A (Subtilisin Carlsberg) and subtilisin B (Subtilisin Novo, Subtilisin BPN')—which have similar enzymatic properties with respect to substrate specificity (1). However, they differ from each other in more than 80 positions in the amino-acid sequence (33), and do not show serologic cross reactions (4, 8, 24). *Flindt* (13), *Pepys et al.* (30) and *Wüthrich & Ott* (38) seem to have been the first to describe pathologic reactions from the respiratory organs as a result of contact with *B. subtilis* enzymes in the detergent industry. Subsequently, skin symptoms due to contact with such enzymes were also described (10, 23, 26).

The aim of the present study was to determine the risk to health from occupational contact with *B. subtilis* enzymes among the employees in two Swedish detergent factories in

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which such enzymes were handled by different techniques. Subtilisin A has been the main constituent of the commercial enzyme preparation used by both factories.

MATERIAL AND METHODS

Production techniques: In one factory (Factory A), enzyme detergents were produced by a "batch" technique, and the enzyme was mixed in powder form with the detergent base. Handling of the enzyme was fairly open and unsheltered giving a mean air-borne enzyme activity of about 5 GU ("glycine units") per m^3 in the respiratory zones of the factory workers (18). In the other factory (Factory B), enzyme detergents were produced with a continuous "process" technique. When production started in 1966, the enzymes were handled without effective protection, but subsequently the factory equipment was successively better shielded as regards the spread of dust. From 1969 the enzyme was encapsulated in non-dusting and non-respirable granules before mixing with the detergent base. The mean air-borne enzyme activities in the respiratory zones of the factory workers were usually less than 1 GU/ m^3 (18).

Material: Thirty-four employees in Factory A (24 men and 10 women) and 30 employees in Factory B (28 men and 2 women) were examined during February to August 1970. They were divided into three exposure groups as follows:

Group A: Persons with direct occupational contact with *B. subtilis* enzymes.

Group B: Persons with indirect occupational contact with *B. subtilis* enzymes; i.e., who did not handle material containing *B. subtilis* enzymes but worked in places where such material was present and where enzyme dust could occur.

Group C: Persons without known direct or indirect occupational contact with *B. subtilis* enzymes.

Methods: Each person was given a physical examination and a careful history was taken with special reference to previous allergic symptoms and diseases of the skin and respiratory system.

Lung function was studied by registration of forced expirograms with a "Vitalograph". Airway conductance was determined with a body plethysmograph according to the method described by *DuBois et al.* (9), and *Brunes & Holmgren* (6). The mean of 10 separate readings was calculated for each person.

Roentgenograms of the lungs were obtained with high-voltage technique at a focus-film distance of 2 m, and an appropriate secondary grid (100 lines/inch, ratio 1:12 focussed at 2 m) was used. Films were taken in frontal, lateral and two oblique projections and were evaluated by the same radiologist, who was

not informed about the degree of exposure to enzymes. The results of the pulmonary roentgenographic findings were divided into four groups:

- I. No changes.
- II. Minor changes, probably of no clinical significance (e.g., calcified primary complex).
- III. Somewhat more pronounced, non-characteristic changes interpreted as residua of healed lung or pleural affections.
- IV. Changes of obscure significance or indicative of active disease in lungs or pleurae.

Intradermal tests with 45 different allergens, and prick test with a standard enzyme reagent*, were performed.

The sera were analysed with reference to the occurrence of enzyme-specific hemagglutinins and IgE-antibodies ("reagins"). Nineteen sera (15 from exposure group A and 4 from exposure group B) were also analysed with reference to subtilisin-specific precipitins. Passive hemagglutination test was performed with sheep erythrocytes treated with tannin (5) or bis-diazo-benzidine (34). On the tannin-treated erythrocytes, crystalline subtilisin A** was adsorbed in the concentrations of 0.01 mg, 0.03 mg, 0.1 mg, 0.3 mg and 1 mg/ml, respectively, and on the bis-diazo-benzidine-treated cells in a concentration of 2 mg/ml. The precipitation test was performed with double diffusion technique according to *Ouchterlony* (29) in the micromodification of *Wadsworth* (35), using crystalline subtilisin A** in concentrations of 0.01 mg, 0.05 mg, 0.1 mg and 1 mg/ml at 4°C and 25°C. The occurrence of enzyme-specific IgE-antibodies was analysed with RAST ("radioallergosorbent test") using the method of *Wide et al.* (37). As coupled antigens, both the commercial enzyme preparation "Alcalase"*** and crystalline subtilisin A** were used.

Student's t-test was used for statistical analysis of numerical variables, and the χ^2 -test with Yates' correction for statistical evaluation of frequency differences between different groups (12).

RESULTS

Respiratory system: Table 1 shows that 50 per cent (25 of 50) of the persons in exposure groups A and B often had irritative symptoms in the respiratory tract at work which resulted in cough. Table 2 shows that 14 per cent (7 of 50) of the enzyme-exposed workers had frequent complaints of

* Provided by Unilever Research Laboratory, Colworth House, Bedford, Great Britain.

** Provided by Novo Industri A/S, Denmark.

TABLE 1.

Table 1. Smokers (≥ 1 Cigarette Daily) and Non-smokers (< 1 Cigarette Daily) with Frequent Cough at Work.

	Exposure group			Total
	A	B	C	
Smokers	11/16	5/10	0/12	16/38 (42 %)
Non-smokers	7/17	2/7	0/2	9/26 (35 %)
Total	18/33	7/17	0/14	25/64
%	55	41	0	39

cough on contact with the enzyme. As regards the cough, the difference between smokers and non-smokers was not significant ($P > 0.1$), nor were there any significant differences between the employees in Factories A and B. The workers in Factory B, however, had noted a decrease in severity as well as in frequency of enzyme-provoked symptoms which paralleled the improvement in dust control. Comparison of *Tables 1 and 2* shows that cough seems more often to be provoked by ingredients of laundry products other than enzymes.

Frequent occurrence of cough unrelated to the work place showed no relationship to exposure to enzyme or to other irritant substances in the detergent factories (*Table 3*). In this respect, there was no difference between the employees

TABLE 2.

Smokers and Non-smokers with Frequent Cough on Occupational Contact with B. Subtilis Enzyme.

	Exposure group			Total
	A	B	C	
Smokers	2/16	3/10	—	5/26 (19 %)
Non-smokers	2/17	0/7	—	2/24 (8 %)
Total	4/33	3/17	—	7/50
%	12	18	—	14

TABLE 3.
Smokers and Non-smokers with Frequent Cough Unrelated to Work.

	Exposure group			Total
	A	B	C	
Smokers	6/16	1/10	3/12	10/38 (26 %)
Non-smokers	1/17	1/7	0/2	2/26 (8 %)
Total	7/33	2/17	3/14	12/64
%	21	12	21	19

in Factories A and B. Only 2 of 12 persons with this symptom were non-smokers, and both had been smokers previously. One of them had stopped smoking because of "cough", and the other because of "trouble with the throat". Of the 12 persons complaining of cough without relation to the work, 9 had chronic bronchitis. This was defined as a cough with expectoration over a period of three months, or as recurrent episodes of this type of at least two weeks duration each, during the previous two years. By this definition chronic bronchitis was not related to the degree of exposure to enzyme or to other irritant substances in the factories.

Both FVC ("forced vital capacity") and MMF ("maximal midexpiratory flow") recorded on forced expirograms, and airway conductance determined with a body plethysmograph, were within normal limits in the men of the exposed groups in both factories (*Tables 4 to 6*). With regard to these variables, no significant differences were observed between the different sub-groups. The mean age of the persons in each group was about 48 years.

Roentgenograms of the lungs: As is apparent from *Table 7*, there were no significant differences between the different exposure groups regarding the incidence of the roentgenological findings, nor were there any significant differences between the employees in Factories A and B. Only one of the persons examined had roentgenographic changes classified as group IV. He had a possible slight

TABLE 4.
FVC (Litre) in the Examined Males.

	Exposure group				Total	
	A	B	C			
n	M $\pm$ S.D.	n	M $\pm$ S.D.	n	M $\pm$ S.D.	n
Factory A	9 4.44 $\pm$ 0.71	7 4.75 $\pm$ 0.46	6 4.39 $\pm$ 1.25	22	4.53 $\pm$ 0.81	
Factory B	12 4.41 $\pm$ 0.53	8 4.66 $\pm$ 0.86	6 4.58 $\pm$ 0.53	26	4.53 $\pm$ 0.63	
Total	21 4.42 $\pm$ 0.60	15 4.70 $\pm$ 0.68	12 4.49 $\pm$ 0.92	48	4.53 $\pm$ 0.71	

n = number of persons examined. M = arithmetic mean. S.D. = standard deviation.

TABLE 5.
MMF (L/sec) in the Examined Males.

	Exposure group				Total	
	A	B	C			
n	M $\pm$ S.D.	n	M $\pm$ S.D.	n	M $\pm$ S.D.	n
Factory A	9 3.02 $\pm$ 1.73	7 3.92 $\pm$ 0.55	6 3.55 $\pm$ 1.69	22	3.45 $\pm$ 1.43	
Factory B	12 3.05 $\pm$ 1.87	8 3.30 $\pm$ 1.08	6 3.63 $\pm$ 1.44	26	3.26 $\pm$ 1.06	
Total	21 3.04 $\pm$ 1.27	15 3.59 $\pm$ 0.90	12 3.59 $\pm$ 1.49	48	3.35 $\pm$ 1.24	

n, M and S.D.: See Table 4.

enlargement of the hilar lymph nodes, but no known exposure to *B. subtilis* enzymes.

Skin: *Table 8* shows that visible skin reactions and subjective skin symptoms connected with work, such as burning and itching, occurred in 47 per cent of all persons examined. The frequency was highest in exposure group A and lowest in exposure group C. The difference between these two groups was significant ($0.05 > P > 0.01$). When in contact with enzyme, 24 per cent of the enzyme-exposed persons noted symptoms of this type (*Table 9*). Most cases showed irritative skin reactions on the wrist, under the collar and on the forehead, especially when the skin was moist. Three of the persons examined developed irritative reactions around and under the nails, when the hands came in contact with concentrated enzyme powder. On one such occasion one of them developed bleeding ulcerations under the nails. When the workers began to protect themselves against contact between enzyme and skin, irritative skin symptoms decreased or disappeared completely. One man got an urticarial rash on the body, when he came into contact with enzymes. This phenomenon was first observed one month after the initial enzyme contact.

A comparison of the information given in *Tables 8 and 9* shows that, in the majority of cases, skin and respiratory symptoms were caused by the ingredients in the laundry products other than *B. subtilis* enzymes. As division of the material into exposure groups is based solely on the degree of enzyme exposure, a comparison of *Tables 8 and 9* also shows that there must be some connection between the level of exposure to enzyme and to other irritating ingredients in laundry products.

Enzyme-provoked symptoms and allergy: In *Table 10* the occurrence of enzyme-provoked symptoms in the skin and respiratory system, and the result of the enzyme-specific prick test are correlated to a family history of allergy in the parents or grand-parents, the occurrence of probably allergic symptoms from the skin and respiratory system prior to occupa-

TABLE 6.
Conductance of the Airways (L/sec/cm H₂O) in the Examined Males.

	Exposure group				Total	
	A		B		C	
	n	M $\pm$ S.D.	n	M $\pm$ S.D.	n	M $\pm$ S.D.
Factory A	9	0.137 $\pm$ 0.059	8	0.137 $\pm$ 0.039	4	0.128 $\pm$ 0.049
Factory B	12	0.131 $\pm$ 0.033	2	0.115 $\pm$ -	0	-
Total	21	0.133 $\pm$ 0.045	10	0.132 $\pm$ 0.039	4	0.128 $\pm$ 0.049
					35	0.132 $\pm$ 0.042

n, M and S.D.: See Table 4.

TABLE 7.
Classification of Pulmonary Roentgenographic changes.

Roentgen group*	Exposure group			Total
	A	B	C	
I	21 (64 %)	12 (70 %)	10 (72 %)	43 (67 %)
II	9 (27 %)	5 (30 %)	2 (14 %)	16 (25 %)
III	3 (9 %)	0 (0 %)	1 (7 %)	4 (6 %)
IV	0 (0 %)	0 (0 %)	1 (7 %)	1 (2 %)
Total	33 (100 %)	17 (100 %)	14 (100 %)	64 (100 %)

* See the text under "Material and Methods".

tional contact with *B. subtilis* enzymes, and to the result of allergy tests. The observations suggest that persons with allergic symptoms prior to occupational contact with *B. subtilis* enzymes ran a greater risk of developing enzyme-provoked symptoms than persons without a history of earlier allergy. In addition, a family history of allergy and one positive reaction, at least, when performing an intradermal test with 45 common allergens usually showed a tendency to increased frequency of enzyme-provoked symptoms, but none of these differences was significant. The correlation between the allergic "stigmata" studied and the enzyme-provoked symptoms was always more pronounced in the respiratory system than in the skin, and significant only in the respiratory system.

If allergic symptoms in the skin or respiratory system prior

TABLE 8.
Skin Symptoms (Itching, Burning and Visible Skin Lesions) at Work.

	Exposure group			Total
	A	B	C	
Factory A	9/18	3/9	2/7	14/34 (41 %)
Factory B	11/15	4/8	1/7	16/30 (53 %)
Total	20/33	7/17	3/14	30/64
%	61	41	21	47

TABLE 9.

Skin Symptoms (Itching, Burning and Visible Skin Lesions) from Contact with B. Subtilis Enzymes at Work.

	Exposure group			Total
	A	B	C	
Factory A	7/18	0/9	—	7/27 (26 %)
Factory B	3/15	2/8	—	5/23 (22 %)
Total	10/33	2/17	—	12/50
%	30	12	—	24

to occupational contact with *B. subtilis* enzyme and/or at least one positive reaction in the intradermal routine test are considered to be indicative of atopy, the enzyme-exposed groups consisted of 18 atopics and 28 non-atopics. There was no significant difference in the frequency of enzyme-provoked symptoms in the skin between atopics and non-atopics. However, although the series is small, there was a probably significantly higher frequency of atopics than non-atopics with enzyme-provoked symptoms in the respiratory system. Enzyme-provoked symptoms in the respiratory system were noted in 65 per cent of the prick-test positive individuals but in only 3 per cent of the prick-test negative individuals.

Serology: Precipitins could not be demonstrated in any of the 19 examined sera from persons exposed to *B. subtilis* enzymes. In contrast, a rabbit anti-"Alcalase" serum* gave definite precipitation lines. The same rabbit antiserum gave a positive reaction to a titer of 1/1280 in passive hemagglutination test with tannin-treated sheep erythrocytes (coating concentration 1 mg/ml). The other sera examined gave a negative reaction in all coating concentrations. However, when bis-diazo-benzidine-treated erythrocytes were used, positive results were common. In *Table 11*, the hemagglutinin titers in the sera from the employees in the detergent factories are compared with corresponding titers from two control groups. "Control group 1" consisted of 60 routine sera which for

* This antiserum was kindly made available by Dr. Lars Belin, Gothenburg.

TABLE 10.

The Relation in the Enzyme-Exposed Groups (Exposure groups A and B) Between the Occurrence of Some Allergic "Stigmata" and the Occurrence of Enzyme-Provoked Symptoms.

	Number	Enzyme-provoked symptoms			Enzyme-specific prick test
		Skin	Respiratory system	Skin or respira- tory system	
Allergic heredity	Yes 6 No 40	2 (33 %) 6 (15 %)	2 (33 %) 4 (10 %)	3 (50 %) 10 (25 %)	1 (17 %) 7 (18 %)
Allergic symptoms from skin or respiratory system prior to occupational contact with B. subtilis enzymes	Yes 9 No 37	3 (33 %) 5 (14 %)	4 (44 %) 2 (5 %)	6 (67 %) 7 (19 %)	4 (44 %) 4 (11 %)
Result of intradermal routine testing (45 allergens)	Pos 14 Neg 32	1 (7 %) 7 (22 %)	4 (29 %) 2 (6 %)	5 (36 %) 8 (25 %)	4 (29 %) 4 (13 %)
Atopy (allergic symptoms prior to enzyme exposure or positive intradermal routine test)	Yes 18 No 28	4 (22 %) 4 (14 %)	5 (28 %) 1 (4 %)	8 (44 %) 5 (18 %)	5 (28 %) 3 (11 %)
Enzyme-specific prick test	Pos 8 Neg 38	2 (25 %) 6 (16 %)	5 (63 %) 1 (3 %)	6 (75 %) 7 (18 %)	— —

(+) 0.1 > P > 0.05. + 0.05 > P > 0.01. ++ 0.01 > P > 0.001. +++ 0.001 > P.

different reasons were sent for examination to the Department of Immunology at the National Bacteriological Laboratory. In these sera no autoantibodies to various autoantigens could be detected. The sera came from persons who were matched against the employees in the detergent factories with reference to sex and age. "Control group II" consisted of sera from 33 employees at the National Bacteriological Laboratory. Here, there was a predominance of women as compared with the other two groups. The frequency of titers $\geq 1/80$ in "control group I" was significantly lower ($0.001 > P$) than in the group of factory employees and "control group II", whereas the difference between the group of factory employees and "control group II" was not significant ($P > 0.1$). If the borderline between positive and negative hemagglutinin titers is located between the dilutions of $1/40$ and $1/80$, positive titers occurred in the sera from 67 per cent (40 of 60) of the employees in the detergent factories (Table 12). The incidence of positive titers was highest in exposure group A and lowest in exposure group C and the difference between these groups was significant ($0.05 > P > 0.01$).

Of 45 sera from the factory employees examined with RAST for demonstration of IgE-antibodies, 5 were positive to both crystalline subtilisin A and the commercial enzyme preparation "Alcalase". The most pronounced reactions were obtained with crystalline subtilisin A. Another two sera were positive to "Alcalase", but negative to crystalline subtilisin A (Table 13).

Enzyme-provoked symptoms and allergy tests: When exposed to enzyme, symptoms from skin or respiratory system had occurred in 13 of 46 (28 per cent) of the persons ranked in exposure groups A and B. In Table 13 the occurrence of some allergic "stigmata" is reported for all persons who reacted positively to a prick test with a standard enzyme reagent. Three of 23 (13 per cent) enzyme-exposed workers from Factory A, and 5 of 23 (21 per cent) enzyme-exposed workers from Factory B, were in this category. All prick-test positive workers from Factory A had noted enzyme-provoked symp-

TABLE 11.
*Enzyme-Specific Hemagglutinin Titers in Sera from the Employees in the
 Detergent Factories as Compared with Two Control Groups.*

	Passive hemagglutinin titer									
	Negative					Positive				
	< 1/10	1/10	1/20	1/40	1/80	1/160	1/320	1/640	1/1280	1/2560
Employees in detergent factories (52 men; 8 women)	1	5	7	7	14	11	10	1	1	3
Control group I (52 men; 8 women)	12	2	13	27	4	1	1	—	—	—
Control group II (6 men; 27 women)	—	2	3	10	7	8	2	1	—	—

TABLE 12.

Frequency of Enzyme-Specific Hemagglutinin Titers ($\geq 1/80$) in Sera from Employees in Detergent Factories.

	Exposure group			Total
	A	B	C	
Factory A	13/14	6/9	4/7	23/30 (77 %)
Factory B	11/15	4/8	2/7	17/30 (57 %)
Total	24/29	10/17	6/14	40/60
%	83	59	43	67

toms; two from the respiratory system and one from the skin. The latter developed urticaria. Three of the workers from Factory B noted enzyme-provoked respiratory symptoms and one of them also complained of enzyme-provoked skin symptoms. Only one of the prick-test positive persons had a definitely negative RAST, and he was one of the two individuals, who had no enzyme-provoked symptoms at all.

In *Table 14* the serologic pattern and occurrence of symptoms in respiratory system and skin are shown for those who have reacted negatively to a prick test with a standard enzyme reagent, but who have nevertheless shown symptoms when they came into contact with *B. subtilis* enzymes in the detergent industry. Six of the 7 persons in this group had skin symptoms, but only one had symptoms from the respiratory system. Skin symptoms were all of an irritative character.

A comparison between *Tables 13 and 14* indicates that there was a difference between prick-test negative individuals with enzyme-provoked symptoms and prick-test positive individuals. The prick-test negative individuals had a preponderance of irritative skin symptoms, while the prick-test positive individuals had a preponderance of symptoms from the respiratory system. RAST positivity was connected with prick-test positivity, whereas hemagglutinin positivity seemed to be independent of the results of both prick test and RAST, and also independent of the occurrence of manifest symptoms due to contact with *B. subtilis* enzymes.

TABLE 13.
*Serologic Findings and Symptoms in Respiratory System and Skin Among Employees in Detergent
 Factories Showing a Positive Enzyme-Specific Prick Test.*

Sex	Exposure group	Factory	Symptoms				Enzyme tests		
			Respiratory organs		Skin		Prick test	RAST**	Hemagglutinin titer
			Before*	After*	Before*	After*			
Male	B	B	+	+	+	+	+	+	1/80 (pos)
Male	A	B	+	+	+	—	(+)	+	1/2560 (pos)
Male	B	A	+	+	—	—	+	+	1/10 (neg)
Female	B	A	+	+	—	—	+	+	1/160 (pos)
Male	A	B	—	+	—	—	+	+	1/320 (pos)
Male	A	A	—	—	—	+	+	+	1/160 (pos)
Male	A	B	—	—	—	—	(+)	+	1/2560 (pos)
Male	A	B	—	—	—	—	—	+	1/160 (pos)

* Allergic symptoms "before" and enzyme-provoked symptoms "after" first occupational exposure to *B. subtilis* enzymes.

** + Positive reaction to both crystalline subtilisin A and "Alcalase".

(+) Positive reaction to "Alcalase", but negative reaction to crystalline subtilisin A.

— Negative reaction to both crystalline subtilisin A and "Alcalase".

TABLE 14.
*Serologic Findings and Symptoms in Respiratory Organs and Skin Among Employees in Detergent
 Factories Showing a Negative Enzyme-Specific Prick Test, but Nevertheless Having Enzyme-
 Provoked Symptoms.*

Sex	Exposure group	Factory	Symptoms				Enzyme tests		
			Respiratory system		Skin		Prick test	RAST	Hemagglutinin titer
			Before*	After*	Before*	After*			
Male	A	B	+	+	—	—	—	—	1/1280 (pos)
Male	A	B	—	—	+	+	—	—	1/2560 (pos)
Male	A	A	—	—	+	+	—	—	1/80 (pos)
Male	A	A	—	—	—	+	—	—	1/80 (pos)
Male	B	A	—	—	—	+	—	—	1/320 (pos)
Male	A	A	—	—	—	+	—	—	1/80 (pos)
Male	B	B	—	—	—	+	—	—	1/20 (neg)

* Allergic symptoms "before" and enzyme-provoked symptoms "after" first occupational exposure to *B. subtilis* enzymes.

DISCUSSION

Irritative symptoms in the respiratory tract resulting in cough are very common in connection with work in detergent factories. Smoking habits obviously have an insignificant influence upon this type of airway irritation and, as a rule, it depends on exposure to substances other than *B. subtilis* enzymes. There is, however, some connection between exposure to such enzymes and exposure to other types of irritants in the occupational environment in question. Enzymes may increase the incidence of such symptoms in the most enzyme-exposed worker categories. On the other hand, in the cases studied, "chronic bronchitis" diagnosed from the histories, as well as frequent cough unconnected with work, are both closely connected with smoking habits, but not with exposure to enzymes or other ingredients of the laundry products. Nor was it possible to demonstrate pulmonary roentgenographic changes referable to enzyme exposure or to discover any signs of persistent airway obstruction among the enzyme-exposed groups. This accords with observations made by *Newhouse et al.* (27), who indeed found signs of airway obstruction during work shifts among enzyme-sensitized individuals, but on repeated examination did not find any tendency to persistent deterioration in ventilatory capacity.

As a rule, respiratory symptoms caused by exposure to *B. subtilis* enzymes depend on allergy. This is commonly due to subtilisin, but the commercial enzyme preparations also contain a number of other allergens (8) of clinical significance. In the present study this is demonstrated by two factory workers who had IgE-antibodies against a commercial enzyme preparation, but lacked demonstrable IgE-antibodies against crystalline subtilisin.

The absence of correlation between prick-test reactivity and occurrence of enzyme-provoked skin symptoms indicates that most of the skin symptoms do not depend on allergy. The only person who showed skin symptoms possibly due to allergy had urticaria. This also suggests that the risk of

developing enzyme allergy is relatively low after skin contact, as compared with inhalation of enzyme powder. If one bears in mind that most enzyme-provoked skin symptoms are irritative in nature and occur mainly on moist areas, it is likely that these symptoms are due to local proteolytic enzyme effects.

In animal experiments it has been possible to induce acute damage to the lungs with intra-alveolar bleeding even with the first exposure to high levels of *B. subtilis* enzymes (7, 17). This is probably due to proteolytic enzyme activity on the moist mucous membranes of the respiratory tract. For many years, before we were aware of the risks connected with enzyme exposure, the concentrated enzyme preparations were often delivered to detergent factories in ordinary sacks. These were often handled in the same manner as sacks filled with—e.g., flour or cement. In view of the animal experiments mentioned it seems probable that the slight changes of the lung function indicating a loss of the elastic coil of the lungs observed by *Gandevia & Mitchell* (16) in a group of heavily enzyme-exposed workers, as well as some of the more dramatic pulmonary symptoms, such as rapidly developing emphysema and the occurrence of nodular densities on chest roentgenograms, described by *Flindt* (13) in his pioneer report but not shown in the present study or in other later examinations (14, 26, 27, 32, 36, 39), are examples of proteolytic enzyme lesions caused by extremely heavy enzyme exposure. In any case, it is obvious that excessive exposure to *B. subtilis* enzymes, for instance in connection with accidents, could create special and very serious health risks even in non-sensitized individuals.

The "Industry Committee on Enzymatic Washing Products" (22) in Great Britain has recommended control of prick-test reactivity both at new-employment examination and, subsequently, at 6 month-intervals for all persons coming in contact with proteolytic enzymes in the detergent industry, if they remain prick-test negative. However, the close correlation between the result of enzyme-specific prick test and the occurrence of enzyme-provoked symptoms in the respiratory sy-

stem does not imply that doing such a test prior to first occupational exposure to detergent enzymes can screen out those more likely to develop enzyme allergy. It is probable that the prick test will nearly always be negative in the absence of previous contact with enzymes that has provoked the formation of enzyme-specific antibodies. The only well-founded indication for performing a prick test with *B. subtilis* enzymes before employment in detergent factories is to detect persons who already have been affected by latent or manifest enzyme allergy. According to *Belin et al.* (2), *Bernstein* (3) and *Fal-leroni & Schwartz* (11), this may occur after exposure to enzyme detergents in the home. The results presented here indicate that it ought to be possible to detect individuals who are allergic to enzymes by taking a specific history of allergy. If special circumstances make more objective methods desirable, *in vitro* tests of the RAST type should be used. For this, hemagglutination and precipitation tests are not suitable. The hemagglutination test will often be positive, even without signs of allergy, although the results obtained indicate that the average hemagglutinin titer in a group of individuals reflects the average level of subtilisin exposure in this group. The precipitation test may be negative in spite of manifest allergy, and positive precipitin reactions have been observed also in individuals without known exposure to subtilisin (3, 13, 21, 30).

It is easy to sensitise experimental animals by intradermal (25) and subcutaneous (28) injections of solutions of *B. subtilis* enzymes. Thus, when a prick-test is done, the tested individual will become exposed to the allergen in such a way as to induce allergy. The ordinary test dose is, of course, very low, but experiments on man show that some individuals may also have a low threshold of sensitization (31). Therefore it is necessary to consider the risk of provoking latent or manifest allergy when performing a prick or intradermal test and also other types of exposure tests. It might be wise to reserve such tests for establishing the diagnosis when there is a manifest disease suspected to depend on enzyme allergy.

There is a tendency for persons showing positive reactions in an intradermal routine test, and with a history of earlier allergic symptoms, to develop enzyme allergy more often than persons showing negative reactions and with negative history of allergy. Although the difference between these two categories is not significant, this tendency accords with observations made by *Greenberg et al.* (19), *Newhouse et al.* (27) and *Weill et al.* (36), who found that atopics develop enzyme allergy more often than non-atopics. This suggests that it is unsuitable to give atopics enzyme-exposed employment. The results obtained indicate that an accurate history focused on presumed allergic diseases is more adequate for detection of atopics than intradermal tests with a large number of allergens. However, it must be noted that in the material presented here almost 40 per cent of all individuals with enzyme-provoked symptoms occur in the group of "non-atopics". This indicates that medical prophylaxis based on detection of atopics may only be of limited value. Medical prophylaxis can only serve as a complement to technical prophylaxis of which the principal aim is to prevent occurrence of air-borne enzyme dust (15, 18).

Among other protective measures, encapsulation of the enzymes in non-dusting and non-respirable granules before mixing with the detergent base will decrease the amount of air-borne enzyme dust in factory environments (15, 18), and probably also in the homes when enzyme detergents are being used. In view of the fact that inhaled enzyme dust brings with it a real risk of sensitization, and exposure to enzyme detergents in the home can sometimes provoke sensitization, it seems desirable that the production of "ungranulated" enzyme detergents should be terminated, not only to protect enzyme-exposed factory workers, but also to protect consumers of laundry products.

SUMMARY

Sixty-four employees in two factories producing laundry products containing *B. subtilis* enzymes were examined with lung-function tests, roentgenograms of the lungs, and a number of allergy tests.

No roentgenological changes or disturbances of lung function referable to enzyme exposure were observed. Irritative symptoms from the skin or respiratory tract referable to the working environment occurred in about 50 per cent of enzyme-exposed workers. Such symptoms often depend on exposure to substances other than *B. subtilis* enzymes, but the enzyme increased the occurrence of such symptoms in the most enzyme-exposed workers. "Chronic bronchitis", diagnosed from the patient's history, and frequent cough unconnected with work were correlated to smoking habits, but not to the degree of enzyme exposure.

The occurrence of enzyme-specific IgE-antibodies determined with RAST is associated with prick-test positivity, while the result of the hemagglutination test is independent of the results of both prick test and RAST, and also independent of the occurrence of manifest symptoms caused by contact with *B. subtilis* enzymes. However, the average hemagglutinin titer in a group of individuals reflects the average level of enzyme exposure in this group.

Enzyme-provoked skin symptoms are, as a rule, due to proteolytic effects, whereas enzyme-provoked respiratory symptoms are usually due to allergy to subtilisin. Other ingredients in commercial enzyme preparations may also possess allergic properties of clinical significance.

Atopics are more prone to develop enzyme allergy than non-atopics. In connection with pre-employment examinations, the atopics may be more effectively distinguished with the help of each patient's history than from the result of intradermal tests with a large number of common allergens. Prick test with subtilisin should be avoided in this situation; it is usually possible to detect enzyme allergics with the help of a careful

patient history. If more objective methods are desirable, in vitro tests of the RAST type are recommended.

The risk of developing enzyme allergy seems to be greater after inhalation of enzyme powder than after contact between enzyme and skin. The need for protection of enzyme-exposed factory workers, as well as the need for protection of consumers of the laundry products, would seem to make it necessary to call a halt to the production of "ungranulated" enzyme detergents.

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