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MEDICAL EXPERIENCE IN ENZYME PRODUCTION

by

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Proteolytic enzymes from *Bacillus subtilis* (subtilisins) have been incorporated in laundry detergents since 1963. The handling of these enzymes in the industry has been reported to cause pathological reactions from the skin and the respiratory tract (2, 3, 5, 10, 11, 12, 14), such effects do not appear to be a major problem among consumers of laundry products. Whereas the occupational enzyme dermatitis is a primary irritant reaction (16), enzyme provoked respiratory symptoms may be of an allergic or non-allergic nature (5).

Proteolytic enzymes were originally supplied as fine powder to the detergent industry. Early reported cases of respiratory symptoms among workers may partly have been due to failure to control dust. Although complaints have decreased (6) following the introduction of encapsulated enzymes and improvement of industrial hygiene, surveying of workers still seems necessary. Investigations on workers from enzyme producing factories are equally important to studies in the detergent industry, but until now only a few data from such investigations have been published, these include a preliminary report on a health survey of 338 enzyme workers presented at the X Scandinavian Congress of Allergology (15) and results of a study on the occupational enzyme dermatitis found in the same group (16). The present report describes

results of the latest investigation on 355 workers from the same two enzyme producing factories. The aim of the study was to determine any present health risk due to occupational contact with Alcalase®, a commercial enzyme preparation derived from subtilisin B. Alcalase is one of the most commonly used enzymes in biological detergents.

MATERIAL AND METHODS

In November 1972 all workers engaged in enzyme production at two danish factories were examined by the company physicians. A careful history was taken with special reference to symptoms of eye, nasal or throat irritation, symptoms of respiratory disease including frequent coughs, shortness of breath, wheezing or chest tightness, and any signs of skin irritation. The history also included family history of atopy and previous signs of asthma, hay-fever or atopic dermatitis. All 355 workers received a general physical examination which included inspection of skin, eyes and throat, lung stetoscopy and a lung function test. The forced expiratory volume in one second (FEV₁) was measured with a VITALOGRAPH spirometer and the results expressed in relation to FEV₁ predicted.

Blood samples were obtained from all workers exposed to enzyme dust and investigated for specific IgE antibodies to *B. subtilis* enzymes by the RAST-test (13). This part of the study included 211 workers. The *in vitro* testing was performed by L. W., University of Uppsala. The same 211 workers all had a chest X-ray. Since 1970 all enzyme workers from these two factories have been questioned and had a physical examination including a lung function study twice a year. Chest X-ray and RAST-test have been performed once a year in all workers exposed to enzyme dust. As earlier studies on 338 workers (15) showed a close correlation between RAST-test and Prick-tests with Alcalase only RAST-test have been included in the continued health survey. The present report includes data of five subsequent analyses of 149 workers examined twice a year during the period November 1970 to November 1972.

Air-borne dust was collected on stationary and portable filters in both factories, and the workers degree of exposure established. 61 workers who had been exposed to an enzyme activity of more than 0.009 mg crystalline pure subtilisin per m³ within the latest six months period were classified as heavily exposed and their data examined separately. Lung functions from 36 workers with a history of more than ten years employment in the enzyme departments were compared with lung functions from 319 workers with less than ten years employment.

All roentgenograms of the lungs were examined at each of the two factories by the same radiologist, who was not aware of the workers degree of enzyme exposure.

TABLE 1.

Details of Complaints among 355 Enzyme Workers interviewed in November 1972.

Complaints	No. of complaints
Eye irritation	3
Nasal- and throat irritation	8
Frequent coughs	10
Shortness of breath and chest tightness	6
Asthma	2*
Skin trouble	15
No complaints	321

* Included among workers complaining of frequent coughs, shortness of breath and chest tightness.

Both workers admitted having asthma also before employment with detergent enzymes.

RESULTS

Table 1 shows that during the period from May 1972 to November 1972 eight workers complained of irritation of the upper respiratory tract while twelve complained of cough on contact with enzyme. Only six complained of shortness of breath or chest tightness, two of these had asthma. However, both workers admitted also having asthma before employment

TABLE 2.

Results of the General Physical Examination of 355 Enzyme Workers in November 1972.

Findings	No. of workers with symptoms
Mild conjunctivitis	3
Redness of oral mucosa	2
Abnormalities in lung stetoscopy, milder changes	10
Abnormalities in lung stetoscopy, moderate changes	1
Dermatitis, mild	16
Dermatitis, moderate	11

TABLE 3.

Findings in Workers with a Positive or Previously Positive RAST test to Alcalase. Lung Function is expressed as FEV₁ in Relation to FEV₁ Predicted.

No.	Months employment	RAST test			Symptoms Respiratory tract	Previous symptoms Respiratory tract	Atopic	Lung function per cent
		1970	1971	1972				
1	9			+	+	—	—	108
2	35		±	+	+	—	—	116
3	258	+	+	+	—	+	+	120
4	28	—	+	+	—	—	—	93
5	37	+	+	+	+	+	+	96
6	41	—	+	+	—	—	—	110
7	10			+	—	—	—	97
8	28	±	+	—	+	+	+	103

with detergent enzymes. Fifteen workers had skin complaints. The results of the general physical examination is presented in Table 2. This shows that in spite of only 15 complaints from the skin, 27 workers displayed signs of enzyme dermatitis. Three workers had a mild conjunctivitis, and eight a light nasal- or throat irritation.

Table 3 shows the findings in eight workers with a positive RAST-test or previous positive RAST-test to Alcalase. Table 4 presents the frequency of positive RAST-tests from 1970 to 1972. Changes in frequency represent a combination of sensitization and fluctuation in labour employment. In 1971–72 an exchange in labour force took place in enzyme production. 125 workers left the factories, among these five RAST-

TABLE 4.

Frequency of Positive RAST Tests to Alcalase 1970 to 1972.

	1970	1971	1972
No of positive tests	9	6	7
Investigated workers	338	158	211
Per cent positive tests	2.7	3.7	3.3

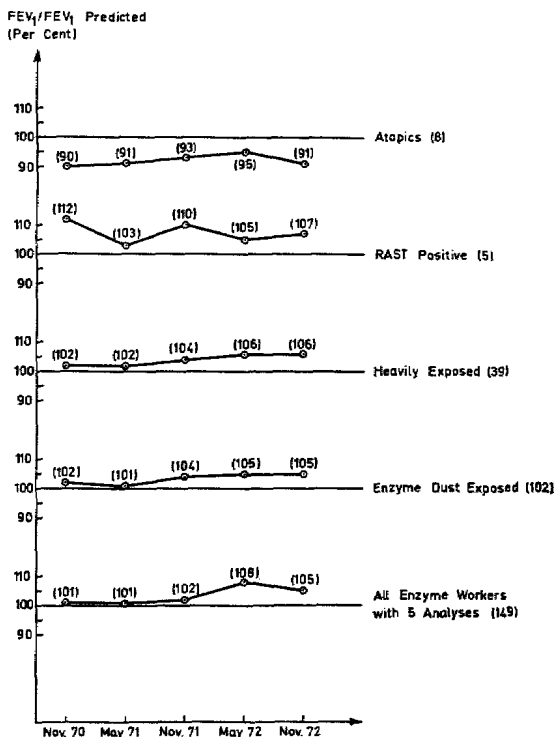


Fig. 1.

Results of lung function studies in various groups of 149 enzyme workers, who have joined five subsequent analyses. RAST-positive workers are no. 2, 3, 4, 5, 6 from Table 3. Results are expressed as forced expiratory volume in 1 sec. (FEV₁) in relation to FEV₁ predicted.

positive workers. The general policy in taking care of sensitized individuals with symptoms has been a transfer to non-dusty enzyme departments or to a non-enzyme department. Also these workers are followed with RAST-tests, lung function studies and chest X-rays in spite of their transfer. Table 3 shows that although sensitized to subtilisins, all eight RAST-positive workers had a lung function within normal

TABLE 5.

Results of Lung Function Studies on 355 Enzyme Workers in November 1972.

	No. of investigated workers	Lung function FEV ₁	± S.E.
		FEV ₁ predicted (per cent)	
All enzyme workers	355	104 ± 1	
Dust exposed	211	105 ± 1	
Heavily exposed	61	103 ± 2	
Over 10 years of enzyme exposure	36	103 ± 3	
Less than 10 years of enzyme exposure	319	104 ± 1	
RAST-positive	8	105 ± 4	
Atopics	16	97 ± 5	
Non-atopics	339	104 ± 1	

range. The number of atopics among RAST-sensitive individuals is high, 38 per cent. The percentage of atopics among all the investigated enzyme workers was 4.5 per cent.

The results of lung function studies can be seen on table 5 and fig. 1. Table 5 shows the results of our latest investigation (November 1972). Fig. 1 only includes data from workers, who have had five subsequent analyses performed from November 1970 to November 1972.

No deterioration of lung function could be demonstrated during the period 1970 to 1972 and no statistical differences could be found between the various investigated groups of workers. Atopics had a slight but not significantly lower lung function in comparison with other enzyme workers. However, not even the atopics showed signs of deteriorating lung function.

Chest X-rays neither showed significant differences between heavily exposed workers and lighter exposed workers, nor were there any significant differences between workers with a high seniority and those newly employed. As a whole only few pathological findings could be seen on the roentgenograms and by the nature of the lesion none of these could be related to enzyme exposure.

DISCUSSION

Our results confirm the earlier findings that atopics are far more likely to develop sensitization to subtilisins than non-atopics (3, 5, 11). It would be advisable therefore, that atopics should not be employed in enzyme production or be subjected to excessive enzyme exposure within the detergent industry. The occurrence of Alcalase specific IgE antibodies is not identical with the occurrence of respiratory tract symptoms. Some sensitized individuals have no symptoms and respiratory tract symptoms may be found, probably on a non-allergic basis (9) in non-sensitized individuals. The risk of developing respiratory problems, however, seems greater among sensitized workers. As mentioned in an earlier report the only patient who developed severe asthma among our workers had a positive RAST-test (15).

The "Industry Committee on Enzymatic Washing Products" in Great Britain has recommended Prick-tests to subtilisins in enzyme workers both at employment and at six months intervals as long as they remain Prick-test negative (8). The previously reported close correlation between results of RAST-tests and Prick-tests to detergent enzymes (15) together with the theoretical risk of sensitization by repeated *in vivo* testing, however, make *in vitro* tests of the RAST type more desirable. Haemagglutination tests and precipitation tests are of no great value. The haemagglutination test may be positive without relation to clinical signs of allergy (5), and positive precipitation reactions have been observed in individuals without known exposure to subtilisins (2, 7).

In our study it was not possible to detect chest X-ray changes or to obtain evidence of persistent airway obstruction due to enzyme exposure. No correlation was found between sensitization to Alcalase and skin symptoms and no worker displayed urticaria. All skin symptoms were of a primary irritant nature. A high percentage of these workers had previously been patch tested with negative results (15) and in

no case were the present lesions typical of allergic contact dermatitis.

The results of this survey show that although irritative symptoms in the respiratory tract and skin troubles may occur in enzyme production, the general health situation of the employees was good. No signs of deterioration of lung function was found, and the percentage of sensitized individuals was low in spite of exposure to enzyme dust.

In view of early experience in plants handling enzymes it seems essential to keep dust levels low. Also accidental excessive exposure to enzyme dust should be avoided. In animal studies (4) lung damage has been demonstrated following high level enzyme exposure. In the detergent industry the preference of encapsulated enzyme products may solve the problem. In enzyme production where the product has not yet reached encapsulation, however, dust exposure may be decreased but not completely eliminated. Naturally major efforts should be continued to be directed to reduce levels of dust so far as is possible. Local extraction of air from all apparatus and working processes which generate dust is important and protective clothing and masks should be provided where necessary.

In the U.S.A. the "American Conference of Governmental Industrial Hygienists" has proposed a threshold limit value (TLV) of 0.0003 mg/m^3 for work with subtilisins as ceiling value (1). Also in Britain requirements have been set up in relation to air-borne subtilisin activity in the work environment (8). These requirements, however, have mainly been based upon early experience from the detergent industry where workers have been exposed to a combination of enzyme dust and detergent. Based upon the present investigation it seems reasonable to propose different TVL for the detergent industry and enzyme production. Continued medical supervision of the health situation in enzyme producing factories should provide a measure of the need of environmental control. Possibly the experience gained in this investigation may be used as a model.

SUMMARY

In a survey 355 workers from two factories producing enzymes for detergents the over all health was found to be good. There was no indication of any deterioration of respiratory functions in either atopic or non-atopic workers, and the skin symptoms were all of a primary irritant nature and not of major incidence. Results obtained by IgE Alcalase specific RAST showed a low level of worker sensitization of 3.3 per cent with a higher sensitivity among atopics.

The ventilatory capacity of both sensitized and non-sensitized workers was good. The average forced expiratory volume in one second expressed in relation to predicted values was 104 per cent. No significant differences in ventilatory capacity were found between heavily exposed and lighter exposed workers. The average ventilatory capacity of sensitized workers was 105 per cent. No signs of deteriorating lung function was found in 149 workers with five analyses performed with six months intervals from November 1970 to November 1972.

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