

# Clinical and immunological responses to occupational exposure to $\alpha$ -amylase in the baking industry

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## Abstract

**$\alpha$ -Amylase is a starch cleaving enzyme often used in the baking industry as a flour additive. It is usually of fungal origin, produced by *Aspergillus oryzae*. One previous report has shown IgE antibodies and positive skin prick test against  $\alpha$ -amylase in asthmatic bakers. This paper describes four  $\alpha$ -amylase sensitised index cases with occupational asthma or rhinitis and the results of a cross sectional study of 20 workers from the same factory who were also exposed to  $\alpha$ -amylase powder. Air sampling detected airborne  $\alpha$ -amylase at a concentration of 0.03 mg/m<sup>3</sup>. Significantly more work related symptoms such as rhinitis and dermatitis were found among the  $\alpha$ -amylase exposed workers compared with referents. A skin prick test to  $\alpha$ -amylase was positive in 30% (6/20) of the exposed workers. Most of the persons showing a positive skin prick test had work related symptoms and were also skin prick test positive to common allergens. Nasal challenge tests with amylase were performed in selected cases and validated three cases of  $\alpha$ -amylase induced rhinitis. Two non-symptomatic workers had precipitins to  $\alpha$ -amylase. Specific IgG antibodies were shown by two further serological techniques. The nature and relevance of these antibodies are currently being studied. It is concluded that  $\alpha$ -amylase powder is a potent occupational sensitiser. Precautions should be taken when handling this allergenic enzyme.**

Allergic asthma and rhinitis caused by occupational

exposure to different microbiologically produced enzymes have been described repeatedly. For example, detergent industry workers were often sensitised to proteolytic enzymes produced by *Bacillus subtilis*.<sup>1-3</sup> Sensitisation to trypsin, papain, and pancreatic extracts has also been reported.<sup>4-6</sup> Immediate type allergy with positive skin prick tests as well as positive radioallergosorbent tests (RAST) have been found, indicating the presence of specific IgE antibodies.<sup>2,3,5</sup>

The ability of industrially used proteolytic enzymes to induce precipitating antibodies and allergic alveolitis has also been discussed. Some precipitins have been supposed to be of non-immunological origin, but IgG antibodies specific to enzyme preparations of *Bacillus subtilis* and papain have been demonstrated by radioimmunological techniques.<sup>2,5,6,7</sup> The presence of IgG antibodies is presumed to be an indication of exposure to enzyme rather than being related to disease.<sup>5</sup>

Industrial fungal  $\alpha$ -amylase is an enzyme produced from a strain of *Aspergillus oryzae*. It hydrolyses glucosidic linkages in starch and is used in the baking industry to supplement flour for yeast leavened dough. This enzyme causes respiratory tract allergy in subjects with positive skin prick test. The first report, in 1979, was from an enzyme handling factory.<sup>8</sup>

Recently, circulating IgE antibodies against  $\alpha$ -amylase have been found in sera from bakers. Positive skin prick tests and bronchial challenges were reported in a few cases.<sup>9,10</sup>

In this paper we present four index cases and a cross sectional survey, all from the same factory in which  $\alpha$ -amylase was handled, confirming the allergenic potency of this enzyme.

This factory produces semimanufactured products for restaurants and bakeries. The employees handle potential industrial allergens such as egg and milk powder, and wheat, malt, and soya flour as well as fungal  $\alpha$ -amylase. About 10 different bread mixes and dough improvers are produced. Fungal  $\alpha$ -amylase from two different sources is added to these mixes. The  $\alpha$ -amylase content of the dough improvers and bread mixes is usually less than 1%.

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Table 1 Index cases

Case No	Diagnosis		Skin prick test			$\alpha$ -Amylase RAST class
	Rhinitis	Asthma	Amylase	Guargum	Common allergens	
1	+	+	++++	+	+	1
2	+	+	++++	-	+	3
3	+	+	+++	-	-	1
4	+	-	++++	-	-	4

Enzyme powder is delivered to the factory in 1 kg bags that are manually emptied into a mixer together with flours and other ingredients. The mixed product is then packed into sacks in an adjacent room.

The first patient of the index cases was seen in 1979. He was a 31 year old man with childhood asthma and eczema who was referred because of suspected work related asthma and rhinoconjunctivitis. Both the skin prick test and RAST against  $\alpha$ -amylase were repeatedly positive. No prick test reactions were found against 10 occupational allergens (guargum gave a positive test) but reactions occurred to several common allergens. The patient's condition improved when exposure to  $\alpha$ -amylase was reduced.

During the subsequent six years three similar patients from this factory appeared at our outpatient unit. Table 1 describes these four index cases.

Because of these findings we conducted the present survey, which aimed to evaluate the health hazard related to  $\alpha$ -amylase and other airborne allergens in this factory.

### Subjects

The survey comprised all 20 persons exposed to fungal  $\alpha$ -amylase in the factory. Sixteen workers were exposed during mixing and packing operations and four during laboratory work. All nine persons manufacturing margarine in another part of the factory building served as referents.

### Methods

All subjects were interviewed by the same experienced allergologist (LB) concerning:

- (1) Previous or present asthma, rhinitis, or skin reactions, and their relation to work.
- (2) Occurrence of the above mentioned diseases among the subject's immediate kin.
- (3) Working history in the factory.
- (4) Smoking habits.

The chest was auscultated. Forced expiratory volume in one second ( $FEV_1$ ) and forced vital capacity (FVC) were measured with a pneumoscope (Jaeger GmbH, Hoechberg, FRG). The highest  $FEV_1$  and FVC of at least two reproducible manoeuvres were recorded and expressed as a percentage of predicted values.<sup>11</sup> Spirometry was repeated five minutes after the inhalation of 0.8 mg salbutamol as a powder mixed with lactose.

Spirometry was considered abnormal if  $FEV_1$  or FVC was less than 80% of the predicted value or if  $FEV_1$  increased more than 15% after inhalation of salbutamol.

### ALLERGY TESTS

Skin prick tests were carried out with the following common inhalation allergens having an extract potency corresponding to 10 000 BU (=10 HEP): birch, timothy, *Dactylis*, and mugwort pollens, *D pteronyssinus*, *D farinae*, *Aspergillus fumigatus*, *Cladosporium*, cat, dog, and horse danders, a control dilution, and histamine dihydrochloride solution at 10 mg/ml.<sup>12</sup> Skin tests were also performed with eight factory products as allergens at a concentration of 1:20 w/v. These were wheat, rye, soya, and malt flour, milk, egg white, whole egg powder, and fungal  $\alpha$ -amylase at 50 mg/ml, all diluted in saline with 50% glycerol and 0.5% phenol. After 15 minutes the weal reaction was graded in comparison with the histamine standard, which was evaluated as a +++-reaction.

Skin prick testing with the amylase solution was also performed in a sample of 25 consecutive patients seen in the Occupational Allergy Unit at the Asthma and Allergy Research Centre. All these tests were negative.

### SEROLOGICAL ANALYSES

Concentration of IgE antibodies to  $\alpha$ -amylase was measured by RAST. A commercial fungal  $\alpha$ -amylase (NOVO industries, Bagsvaerd, Denmark) was conjugated to cyanogen bromide activated cellulose discs according to standard procedures. A <sup>125</sup>I labelled anti-IgE preparation (Pharmacia, Uppsala, Sweden) employed in our common RAST routine, was used as a tracer.<sup>13</sup> The lower limit for a positive test was defined according to the Phadebas RAST reference system (Pharmacia Diagnostics, Uppsala, Sweden).

The concentrations of total circulating IgE were determined by the paper radioimmunosorbent test (PRIST; Pharmacia, Uppsala, Sweden) and were expressed as kU/l serum. Values higher than 100 kU/l were regarded as raised.

Precipitating antibodies were assayed by diffusing 15  $\mu$ l fungal  $\alpha$ -amylase at a concentration of 1.0 mg/ml against a serum well containing 0.2 ml human serum in agarose gel for three days.

Countercurrent electrophoresis in agarose at pH 8.6, a more sensitive gel based analytical method for the detection of precipitating antibodies, was employed using  $\alpha$ -amylase at 10 mg/ml and a twofold dilution series of each worker's serum.

Concentration of IgG antibodies to  $\alpha$ -amylase was also measured by an enzyme linked immunosorbent assay (ELISA) technique employing peroxidase conjugated rabbit antihuman IgG (Dako-Immunglobulins AS, Copenhagen, Denmark). The absorbance at 450 nm of a serum dilution of 1:20 was recorded. An absorbance of more than 0.5 was defined as positive.

#### CHALLENGE TESTS

All workers with positive skin prick tests to  $\alpha$ -amylase (except one, excluded due to pregnancy) underwent nasal challenge tests with fungal amylase. After spraying a saline aerosol in one nostril, 0.2 ml amylase solution at a concentration of 1.0 mg/ml was sprayed in the same nostril. The challenge continued with a 10-fold increase of concentration until a positive reaction was achieved or until a concentration of 100 mg/ml was reached.

The response was measured by weighing the amount of nasal secretion, by recording subjective symptoms such as nasal blockage, itching and number of sneezes, and by posterior rhinomanometry. Rhinomanometric results were expressed as the gradient (in degrees) of the flow pressure curve generated during tidal expiration through the challenged nasal cavity. The challenge was regarded as positive if 0.1 g or more of nasal secretion was found together with an increase of more than 50% in the measured nasal resistance.<sup>14,15</sup>

#### EXPOSURE

The aims of the exposure measurements were limited to assess the total dust concentration and detection of airborne fungal  $\alpha$ -amylase during mixing and packing.

Dust exposure was measured by a sampler carried by one worker during packing of mixed dough improver and by another stationary sampler placed near to where the amylase sacks were emptied into

the mixer (the worker emptying the sacks refused to carry a sampler). Two samples were taken at each location. Dust was collected on cellulose acetate filters with a pore size of 0.8  $\mu$ m at a flow rate of 2.0 l/min. Sampling time varied from 30 to 60 minutes, covering the entire mixing and packing period of that day.

The total dust exposure was measured by weighing the filters. The  $\alpha$ -amylase activity of the dust was calculated with the Phadebas amylase test (Pharmacia, Uppsala, Sweden). The test was adapted to air samples by eluting the filters in 10 ml distilled H<sub>2</sub>O for one hour at +8°C. A pellet of coloured starch was added to the solution; the absorbance at 620 nm was recorded and compared with a standard curve.

Exposure measurements showed total dust concentrations of 6.7 and 10 mg/m<sup>3</sup> during packing. The  $\alpha$ -amylase content of the dust corresponded to an air concentration of 0.03 mg/m<sup>3</sup>. This content accords with the proportion of  $\alpha$ -amylase in the mixed product. Air sampling during mixing showed total dust concentrations of 2.8 and 2.3 mg/m<sup>3</sup>. This dust did not contain detectable  $\alpha$ -amylase activity.

#### DATA ANALYSIS

A statistical comparison of the clinical and immunological findings was done with Fisher's exact test (two tailed). Comparisons between FVC and FEV<sub>1</sub> values used Student's *t* test. The correlation between amylase-IgG countercurrent electrophoresis titres and ELISA-absorbance was estimated by Pearson's coefficient of correlation.

#### Results

Work related symptoms such as rhinitis and dermatitis were reported by 11 of the 20 amylase exposed workers (table 2). Symptoms suggesting work related asthma were not reported. The occurrence of work related symptoms was significantly more common in the exposed subjects than in the referents (*p* < 0.01). Six of the exposed workers showed a positive skin prick test to amylase; two of them were also positive to malt flour and one to several different flours.

Spirometry was normal in all but five cases with previously known disease. The means of FEV<sub>1</sub> and

Table 2 Clinical and immunological findings of the cross sectional study

	No of exposed subjects		No of referents
	With symptoms (n = 11)	Without symptoms (n = 9)	Without symptoms (n = 9)
Positive skin prick test:			
$\alpha$ -Amylase	4	2	1
Malt	2	1	0
Flours	1	0	0
Common allergens	4	1	2
Raised total IgE concentration	3	1	2
Positive amylase RAST	0	1	1
Positive amylase IgG	0	3	0

Table 3 Findings in  $\alpha$ -amylase skin prick test positive workers

	No of exposed subjects (n=6)	No of referents (n=1)
Asthma	0	0
Rhinitis	3	0
Dermatitis	1	0
Positive skin prick test to common allergens	3	1
Raised total IgE concentration	3	1

FVC as per cent of predicted values were 95 and 96% in the exposed group and 100 and 96% in the referents, a difference not statistically significant.

Table 3 describes workers with positive skin prick tests to  $\alpha$ -amylase. All three workers with positive skin prick tests to common allergens as well as to  $\alpha$ -amylase had raised total IgE concentrations whereas those with positive skin prick tests only to amylase had normal IgE concentrations. Smoking habits were not different in those workers who were skin prick test positive to  $\alpha$ -amylase and those who were not.

By immunodiffusion analysis, precipitating antibodies to  $\alpha$ -amylase were demonstrated in the sera of two exposed non-symptomatic workers. Countercurrent electrophoresis showed titres of 1:64 and 1:128 in those two workers. One other serum, which showed a weak precipitin reaction, was positive with a titre of 1:4. These three sera were also positive in an ELISA IgG antibody test using  $\alpha$ -amylase as a coating antigen. Four more sera were positive only in the countercurrent electrophoresis with titres between 1:1 and 1:8. The figure shows the good agreement between the countercurrent electrophoresis and the ELISA test results ( $r = 0.82$ ,  $p < 0.001$ ).

$\alpha$ -Amylase nasal challenge tests were regarded as clearly positive in three out of six tested workers (table 4).

### Discussion

The experience of four index cases from one factory appearing with asthma, or rhinitis, or both at the outpatients' department gave reason to suspect that fungal  $\alpha$ -amylase powder is a potent occupational allergen. These suspicions have now been confirmed by results from this cross sectional study of workers from the same factory.

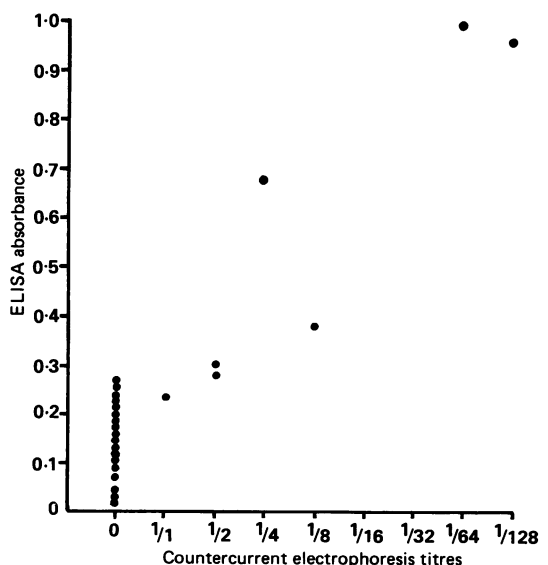
From the index cases we realised that previous severe allergic reaction to  $\alpha$ -amylase—that is, asthma—forced the affected workers to leave the  $\alpha$ -amylase exposed jobs. Accordingly, it could be anticipated that a cross sectional study would disclose only upper respiratory tract symptoms of sensitisation.

We found a significantly higher prevalence of nasal

and skin symptoms among workers exposed to  $\alpha$ -amylase compared with referents. These  $\alpha$ -amylase exposed workers were temporarily exposed to high total dust concentrations during mixing and packing. Some of the symptoms might therefore be caused by non-specific irritative mechanisms. These operations, however, occupy only a minor part of the working day and the dust exposure over the whole eight hour working day would be much lower and thus unlikely to cause all the symptoms recorded. In our view, the presence of positive skin prick test to  $\alpha$ -amylase is evidence of an IgE mediated symptomatology in many of the workers.

The highly concentrated  $\alpha$ -amylase extract that was used for skin prick test probably promoted a high test sensitivity. Despite this, no positive reactions occurred among the skin prick tested controls from the Occupational Allergy Unit. The discrepancy between the number of skin prick test positive and RAST positive persons would indicate a lower test sensitivity in our RAST. It is possible that optimisation of the conjugation of RAST discs would make the two test systems more comparable. We think that workers with positive skin prick test to fungal  $\alpha$ -amylase are truly sensitised, although some of them have not yet developed clinical symptoms.

The pathogenetic significance of the IgG antibodies to  $\alpha$ -amylase is unclear. None of the three exposed workers with antibodies detectable by all three immunological techniques employed, reported work related symptoms, and their spirometric recordings were normal. Two of them were non-



Correlation between  $\alpha$ -amylase antibody determination using countercurrent electrophoresis and IgG-ELISA absorbance (correlation coefficient  $r = 0.82$ ,  $p < 0.001$ ).

Table 4 Outcome of nasal challenge tests with amylase in skin prick test positive workers

	Subject No					
	1	2	3	4	5	6
Occupational amylase exposure	+	+	+	+	+	-
Nasal secretion (g)	0.9	0.1	0.6	0	0.1	0
Per cent increase in nasal resistance	117	64	165	11	34	50
Subjective symptoms	-	+	+	+	+	-
Challenge result	+	+	+	-	-	-

smokers. The two workers with a strong precipitin reaction are currently undergoing further examination.

With this study design we validated three cases of fungal  $\alpha$ -amylase induced rhinitis. The diagnosis was based on a clinical history, positive skin prick test, and a positive nasal challenge to  $\alpha$ -amylase.

This study also provides a possibility to compare the allergenic potency of  $\alpha$ -amylase with the potency of well known flour allergens, because all workers exposed to  $\alpha$ -amylase are also exposed to different flours. In fact the bread mixes and dough improvers mainly consist of flour. The exposure to flour should be more than 100 times greater than that of  $\alpha$ -amylase. Nevertheless, a positive skin prick test reaction to cereal extracts was seen in only three workers compared with twice as many workers who were positive to fungal  $\alpha$ -amylase.

In summary the study showed a high prevalence of rhinitis and dermatitis among workers producing  $\alpha$ -amylase containing "bread improvers". It is concluded that  $\alpha$ -amylase is a potent sensitiser, much more potent than cereal flours. It is assumed that bakers are also at risk of being sensitised and precautions should be taken when handling  $\alpha$ -amylase powder.

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- Greenberg M, Milne JF, Watt A. Survey of workers exposed to dusts containing derivatives of *Bacillus subtilis*. *Br Med J* 1970;2:629-33.
- Pepys J, Wells ID, D'Souza MF, Greenberg M. Clinical and immunological responses to enzymes of *Bacillus subtilis* in factory workers and consumers. *Clin Allergy* 1973;3:143-60.
- Belin L, Norman P. Diagnostic tests in the skin and serum of workers sensitized to *Bacillus subtilis* enzymes. *Clin Allergy* 1977;7:55-68.
- Colten HR, Polakoff PL, Weinstein SF, Strieder D. Immediate hypersensitivity to hog trypsin resulting from industrial exposure. *N Engl J Med* 1975;20:1050-3.
- Tarlo SM, Shaikh W, Bell B, et al. Papain-induced allergic reactions. *Clin Allergy* 1978;8:207-15.
- Weissmann K-J, Baur X. Occupational lung disease following long-term inhalation of pancreatic extracts. *Eur J Respir Dis* 1985;66:13-20.
- How MJ, Cambridge GW. Prick-test and serological tests in the diagnosis of allergic reactivity to enzymes used in washing products. *Br J Ind Med* 1971;28:303-7.
- Flindt MLH. Allergy to  $\alpha$ -amylase and papain. *Lancet* 1979; i:1407-8.
- Baur X, Fruhmans G, Haug B, Rasche B, Reiher W, Weiss W. Role of *Aspergillus amylase* in baker's asthma. *Lancet* 1986; i:43.
- Baur X, Weiss W, Saner W, et al. Backmittel als mitursache des Bäckerasthmas. *Deutsche Medizinische Wochenschrift* 1988; 113:1275-8.
- Berglund E, Birath G, Bjure J, et al. Spirometric studies in normal subjects. *Acta Med Scand* 1963;73:185-91.
- Aas K, Belin L. Standardization of diagnostic work in allergy. *Acta Allergologica* 1972;27:439-68.
- Ceska M, Eriksson R, Varga JM. Radioimmunosorbent assay of allergens. *J Allergy Clin Immunol* 1972;49:1-9.
- Wihl J-Å. *Assessment of nasal hyperreactivity*. Malmö: University of Lund, 1986:95. (Thesis.)
- Clement PAR. Committee report on standardization of rhinomanometry. *Rhinology* 1984;22:151-5.

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