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Occupational asthma caused by α -amylase inhalation: Clinical and immunologic findings and bronchial response patterns

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Inhalation of dust from different enzymes can be the cause of occupational asthma in exposed workers. α -Amylase, derived from Aspergillus oryzae, is one of these enzymes, although there are few studies in the medical literature that refer to its allergologic properties and to clinical studies in sensitized patients. The results obtained in a study performed in 83 pharmaceutical-industry workers exposed to powdered α -amylase are described in this article. The existence of sensitization to this enzyme was demonstrated in 26 of the workers by positive skin tests. Specific IgE values were significantly higher in workers with positive skin tests than in workers with negative skin tests ($p < 0.001$). The bronchial provocation test with α -amylase was positive in six of the 14 patients challenged, and only immediate bronchial responses were observed; the same type of response was obtained by nasal provocation. One of the workers had a positive response to oral provocation with this enzyme, presenting abdominal, skin, and respiratory symptoms a few minutes after ingestion. Consequently, we consider that the bronchial asthma presented by the workers was due to an immediate-type, IgE-dependent, immunologic mechanism. (J ALLERGY CLIN IMMUNOL 1992;89:118-25.)

Key words: α -Amylase, Aspergillus oryzae, enzymes, bronchial provocation test, occupational asthma

Some enzymes, independently of their origin, are of increasing importance in allergic pathology, particularly in the working environment in which they are often the etiologic agents of occupational asthma.

Various authors have described cases of rhinitis and BA of allergic mechanism in workers exposed to the

Abbreviations used

BA:	Bronchial asthma
PBS:	Phosphate-buffered saline
REIA:	Reverse-enzyme immunoassay
OD:	Optical density
RT:	Room temperature

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dust of diverse enzymes, demonstrating their great sensitizing capacity.¹⁻⁹

In the last few years, interest has been demonstrated in α -amylase, a glycolytic enzyme with a molecular weight of 51,000 daltons. It can be obtained from different sources, such as human saliva, pig pancreas, *Bacillus subtilis*, *Aspergillus oryzae*, etc.

Because of its enzymatic activity (it catalyzes carbohydrate hydrolysis in glycoside groups 1 to 4), it is used as an additive to flour in bakeries and flour mills, as well as in the pharmaceutical industry in which it is used in the composition of some medicines for dyspepsia.

The first description of α -amylase allergy was given by Flindt¹⁰ who reported the first cases of allergic occupational asthma to this enzyme in exposed workers. Diagnosis was based on the clinical history and skin test results. Some years later, our group presented a short communication that described two cases of occupational asthma caused by α -amylase inhalation; we demonstrated the existence of an IgE-dependent, immunologic mechanism responsible for the clinical picture by means of positive skin tests and the finding of specific IgE antibodies, as well as by the existence of an immediate positive response to the bronchial provocation test.¹¹ In one of the previously cited articles, Wiessmann and Baur⁹ demonstrated that pancreatic α -amylase was responsible by a type I hypersensitivity mechanism for the allergic respiratory symptoms in a group of workers exposed to pancreatic extracts.

The role of α -amylase in baker's asthma has recently aroused growing interest. The studies available reveal that, in many cases, the causal agent is not the cereal flour but the α -amylase used as an additive.¹²⁻¹⁴

In our present work, we refer to the results of a study of pharmaceutical-industry workers exposed to the dust of diverse enzymes, including α -amylase of mycogenic origin (*A. oryzae*), who were found to have respiratory symptoms in relation to their work environment.

MATERIAL AND METHODS

Patients

A survey was made of 83 pharmaceutical workers exposed to powdered *Aspergillus* α -amylase. A clinical questionnaire was distributed among the workers to collect any existence of respiratory, nasal, or bronchial symptoms, the possible relationship with the working environment, the symptoms observed in vacation periods or during absence from work, the existence of allergic history, level of exposure, etc. Peak expiratory flow rates at work were only occasionally recorded.

All workers had skin tests, as described below, and a serum sample was obtained to perform the in vitro tests.

Skin prick tests

Antigen preparation. α -Amylase of mycogenic origin (*A. oryzae*) was provided by the pharmaceutical company.

The allergenic extract was prepared by dissolving 2 gm of α -amylase in 20 ml of PBS, pH 7.3, at RT. After the

solution was stirred for 60 minutes and passed through filter paper, the solution was dialyzed in PBS for 24 hours and then passed through a 0.22 μ m Millipore filter (Millipore Corp., Bedford, Mass.) for sterilization. This solution had a final concentration of 10% wt/vol and was considered the undiluted extract. Tenfold dilutions were made for skin and inhalation tests.

Skin test protocol. The prick test technique was used. Dilutions were made from the undiluted extract to 1:10, 1:100, 1:1000, and 1:10,000 in PBS. Skin tests were started with the highest dilution (1:10,000). Responses were examined at 15 minutes and then at 6 hours to detect possible late reactions. Histamine phosphate at 1:100 wt/vol and PBS were used as positive and negative controls, respectively.

Skin prick tests were also done with a battery of common inhalant allergens, including house dust mites, grass and olive-tree pollen, and dog and cat dander (Alergia e Inmunología Abelló, Madrid, Spain). *A. fumigatus*, *A. oryzae*, *A. niger*, *Mucor racemosus*, *Cladosporium herbarum*, and *Alternaria tenuis* extracts (Hollister-Stier Laboratories, Spokane, Wash.) were also tested.

Fifty allergic and 50 nonallergic patients from our clinic, who served as a control group, were tested with the maximum concentration of α -amylase extract.

In vitro tests

Specific IgE determination (REIA assay). The determination of specific IgE antibodies was performed by use of an REIA, as previously described for other antigens,^{7, 15, 16} which consisted basically of the following stages: (1) One milligram of periodate-oxidized peroxidase was mixed with 150 μ l of α -amylase (3 mg/ml) and 100 μ l of carbonate-bicarbonate buffer (1 mol/L, pH 9.5) and kept at 4° C for 18 hours. (2) Then, 2 mg of sodium borohydride was added, and the solution was dialyzed against PBS at 4° C for 24 hours. (3) After dialysis, 1% bovine serum albumin in PBS containing 25% glycerol was added to a final volume of 2 ml. (4) This final solution was aliquoted and stored at 4° C.

Microplates (M 24 AR, Dynatech Laboratories, Inc., Alexandria, Va.) were coated with monospecific antihuman IgE (Tago Inc., Burlingame, Calif.), as previously described.¹⁷ Then, 0.05 ml/well of patient's serum mixed with 0.05 ml of PBS-Tween was incubated for 18 hours at 4° C during constant shaking.

After several washes, 200 μ l of a dilution of the allergen-enzyme conjugate made in PBS-Tween, 25% calf serum, containing 1 μ g of peroxidase, was added to each well and left shaking for 1 hour at room temperature. Then, after various washes with PBS-Tween and tap water, the substrate (0.1 ml/well) was added and left for 30 minutes. The reaction was arrested with 4 N H₂SO₄ (0.1 ml/well), and the plates were read on a Titertek Multiskan (Flow Laboratories, Irvine, Scotland) at 492 nm. The substrate was composed of 1,2-phenylenediamine (2 mg/ml) citrate-phosphate buffer (0.1 mol/L, pH 5; 0.003% H₂O₂).

REIA inhibition. This method was used to investigate the specificity of the α -amylase REIA. For this assay, the same

amount of α -amylase/peroxidase conjugate was mixed with increasing amounts of different allergens. The method previously described for other antigens was followed.^{7,18} Fungal α -amylase, *A. oryzae* (the fungus that produces α -amylase), and pig pancreas α -amylase (Sigma Chemical Co., St. Louis, Mo.) were used as allergens.

Specific IgG determination. This procedure was performed with an indirect ELISA. The same type of microplates as for the REIA method was used. The plates were incubated with 0.1 ml of α -amylase (10 μ g/ml in PBS) for 18 hours at RT. Plates were then washed with PBS-Tween, and 0.25 ml of 2% bovine serum albumin in PBS was added to each well and maintained at 37°C for 1 hour. After an additional wash with PBS-Tween, 0.1 ml of patient's serum at 1:500 in PBS-Tween was added to each well. The plates were incubated for 1 hour at RT during constant shaking. After plates were washed with PBS-Tween, 0.1 ml/well of goat antihuman IgG (Tago Inc.), diluted to 1:4000 in 0.5% PBS-Tween, and 25% fetal calf serum, labeled with peroxidase, was added. The plates were incubated for 1 hour at RT during constant shaking. The plates were washed with PBS-Tween, and finally, the same substrate used in the REIA was added. The reaction was then arrested with 0.1 ml/well of 4N H₂SO₄, and the plates were read on a Titertek Multiskan at 492 nm.

Inhalation provocation tests

The workers who were first observed with respiratory symptoms and positive skin tests to α -amylase and voluntarily accepted further study had specific nasal and/or bronchial provocation tests. Methacholine inhalation tests were not routinely done. All patients had been free of α -amylase dust exposure for 1 week previous to the time the inhalation challenges were done. The following methods were used:

1. **Bronchial provocation test.** Powdered α -amylase from *A. oryzae* (provided by the pharmaceutical laboratory) was mixed with lactose in identical capsules, as described in previous publications.^{7,18} Three batches of capsules were prepared. Each capsule in the first batch contained 99.90 mg of lactose and 0.10 mg of α -amylase. The capsules in the second batch contained 99.75 mg of lactose and 0.25 mg of α -amylase. The third batch contained 99.50 mg of lactose and 0.50 mg of α -amylase. Identical capsules containing 100 mg of lactose were used as placebo.

The patient inhaled the capsule contents through a turboinhaler (Spinhaler, Fisons Corp. Loughborough, England), starting with the placebo and continuing with the capsule with the least concentration of allergen until the maximum dose was reached. The test was undertaken when the patients were absent from work, asymptomatic, and not taking any medication that could affect the test results. There was a control day with exposure to only the lactose powder, and then the different doses of α -amylase were inhaled at 24-hour intervals. Bronchial provocation tests were stopped when a positive response was obtained, which was considered to be a 20% or more fall in FEV₁ of the previously obtained baseline. The tests were conducted with a Vital-

ograph spirometer (Vitalograph Ltd., Buckingham, U.K.). A patient was considered suitable for the test if his or her basal FEV₁ value was $\geq 80\%$ of the predicted value. Once the test was started, measurements were taken at intervals of 5 minutes for the first half hour, then at 60 minutes, and later, at intervals of 1 hour for 8 hours. Then, hourly peak flow measurements were performed (respecting sleeping periods) for 24 hours after each challenge to evaluate the late response. This response was considered a 35% or more fall in peak expiratory flow rate from the baseline.

Ten nonexposed patients with asthma and 10 asymptomatic exposed workers were also challenged with the maximum concentration of 0.50 mg of α -amylase.

2. **Nasal provocation test.** A solution of α -amylase was prepared at a concentration of 1:100 wt/vol, as previously mentioned; 0.05 ml of the solution was inserted up one nostril and 0.05 ml of PBS up the other nostril. Symptoms were observed after 30 minutes and then at intervals of 1 hour for 8 hours. The evaluation of this test was done by assessing nonventilatory parameters, such as pruritus, nasal stuffiness, spontaneous sneezing, and nasal secretion.

Ten nonexposed patients with allergic rhinitis and 10 asymptomatic exposed workers had the same test.

Oral challenge test

To determine whether there was some type of allergic response after ingestion of α -amylase, a double-blind, placebo-controlled, oral challenge test was performed on some workers. The test was done by administering increasing quantities of α -amylase in capsules containing 9 mg of lactose and 1 mg of α -amylase, 8 mg of lactose and 2 mg of α -amylase, and 5 mg of lactose and 5 mg of α -amylase. Finally, the maximum dose administered was 10 mg of α -amylase. Identical capsules containing 10 mg of lactose were used as placebo.

Patients received only lactose on the first day; then, one dose of α -amylase per day was administered. After each oral challenge, the patient was kept in observation for 8 hours. Spirometric controls were performed before and after the oral provocation, with the same steps as in the bronchial provocation test. During the period of observation, the appearance of nasal or bronchial symptoms and the presence of skin or gastrointestinal symptoms were assessed.

The maximum dose of 10 mg of α -amylase was administered to 10 nonexposed patients from our clinic and to 10 asymptomatic exposed workers who served as control subjects.

RESULTS

Clinical questionnaire

Of the 83 workers studied, 80 answered the clinical questionnaire. Of these workers, 47 had nasal symptoms suggestive of allergic rhinitis (58.7%), whereas 24 of the 47 workers had symptoms suggestive of BA (30%).

The age of the patients ranged from 19 to 63 years with a mean of 29.8 years; 31.3% of the workers had

a history of allergy. There was a predominance of female (88.7%) over male workers (13.3%).

The length of time the patients had been working in the pharmaceutical industry ranged from 3 to 17 years with a mean of 9.5 years. The mean time of symptoms in the symptomatic individuals was 3.9 years, with extreme values ranging from 1 to 11 years.

The level of exposure to α -amylase at work was intense (constant) in 38 patients (47.5%), intermediate (intermittent and frequent) in 27 patients (33.7%), and low (intermittent and occasional) in 15 patients (18.8%).

Skin tests

The skin prick tests with α -amylase were positive in 26 workers (31.3%). Twenty of the workers had symptoms of rhinitis and/or BA, whereas six of the workers with positive skin tests were asymptomatic.

In the skin tests performed with a panel of common allergens, eight patients (9.6%) were positive to grass pollens, four to house dust mites, and two patients, to cat dander. There were no positive results with environmental fungi, including different *Aspergillus* spp. among them *A. oryzae*.

The skin tests with the maximum concentration of α -amylase were negative in the control group, indicating the nonexistence of irritating activity.

In vitro tests

Specific IgE. Specific IgE was determined according to the method described in 83 cases. The mean value obtained was 0.632 ± 1.943 OD ($\bar{x} + SD$) and with extreme values of 0.011 and 12.968 OD. The specific IgE level was determined in 10 nonexposed allergic patients, with a mean value of 0.060 ± 0.030 OD.

Two groups were formed of the workers, one with positive skin tests and the other group with negative skin tests. The mean values of specific IgE were compared in both groups to determine any significant differences. These results and the statistically significant differences are presented in Table I.

Positive specific IgE values were considered as ≥ 0.150 OD, that is, equal or higher than the mean value of the control group + 3 SD. On correlating the positive skin test patients with the IgE-positive patients, a statistically significant association ($p < 0.001$, chi-square test) was found (Fig. 1).

REIA inhibition. The existence of cross-reactivity between α -amylase and *A. oryzae* and between fungal α -amylase and pig pancreas α -amylase was not found. The results of the REIA-inhibition assay are presented in Fig. 2.

Specific IgG. The mean value of specific IgG in the

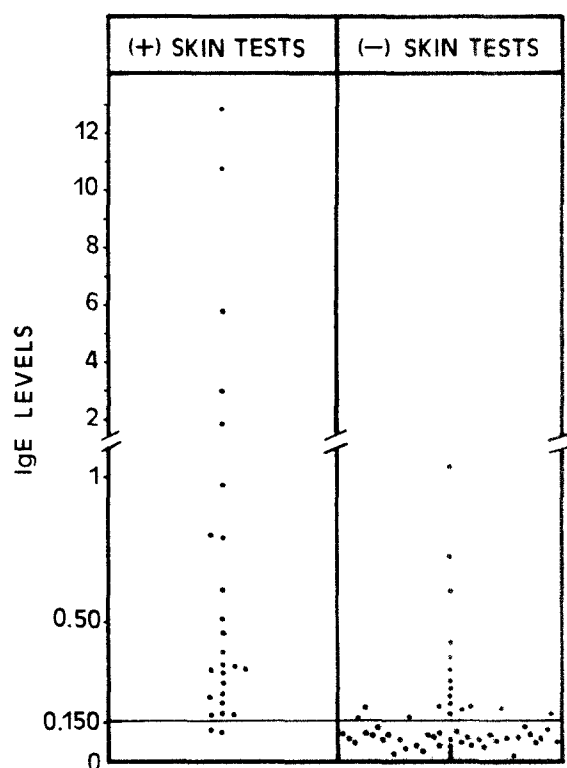


FIG. 1. Relationship between specific IgE levels and skin tests ($p < 0.001$, chi-square test). Each point represents an individual patient. Values >0.150 OD were considered positive.

TABLE I. Specific IgE to α -amylase values

Patients	Mean value*	SD
Skin tests		
Positive (N = 26)	1.677	3.318
Negative (N = 57)	0.173	0.206
Control group (N = 10)	0.060	0.030

* $p < 0.001$.

83 workers was 0.657 ± 0.406 OD, with extreme values of 0.161 and 2.000 OD. The mean value of the control group was 0.050 ± 0.060 OD.

The IgG values in the group with positive and negative skin tests, summarized in Table II, demonstrated no significant differences. Specific IgG values did not correlate with any symptom.

Inhalation provocation tests

Bronchial provocation test. Six (42.9%) of the 14 individuals tested (who gave informed consent) had positive results. All individuals presented immediate-

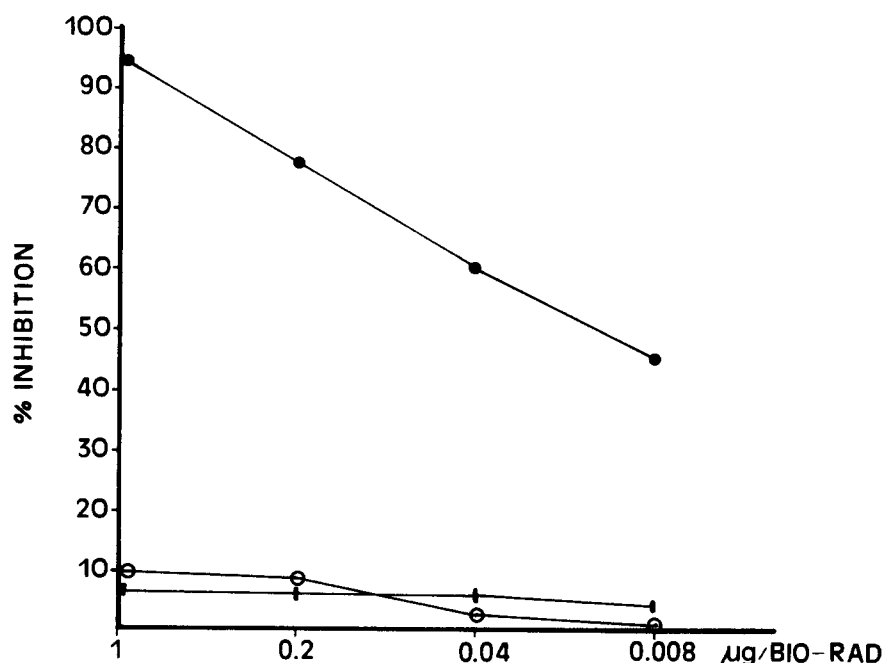


FIG. 2. REIA inhibition performed coincubating labeled α -amylase (1 μ g per well) with α -amylase (●), *A. oryzae* (○), and pig pancreas α -amylase (◻).

TABLE II. Specific IgG values

Patients	Mean value	SD
Skin tests		
Positive (N = 26)	0.678	0.361
Negative (N = 57)	0.648	0.427
Control group (N = 10)	0.050	0.060

type responses (Figs. 3 and 4). In two cases, the tests were repeated after 40 mg of disodium chromoglycate was administered, partially inhibiting the response in one of the individuals (Fig. 3). No dual or late reactions were observed.

None of the control subjects had a positive response, even with the maximum dose of 0.50 mg.

Nasal provocation test. Six (54.5%) of the 11 individuals tested had positive results. Nasal response was immediate in all of the individuals. In the six workers with positive nasal provocation test, the test was positive in two, negative in two, and was not performed on the other two individuals. None of the control subjects had a positive response.

In summary, 18 inhalation, bronchial, or nasal provocation tests were performed, and some of the tests were positive in 10 cases (55.5%).

Oral provocation test. Oral provocation, with the method previously described, was performed in five workers with positive skin tests.

With the maximum dose of 10 mg, one worker had a positive response, generalized pruritus, urticaria, tachycardia, wheezing, and dyspnea. There was a 30% fall in FEV₁ from the baseline at 15 minutes (Fig. 5). The patient recovered after treatment with 0.5 ml of subcutaneous adrenaline, 1:1000. However, this subject does not experience symptoms when he eats bread. None of the control subjects had a positive response.

DISCUSSION

There are not many studies of allergic occupational asthma caused by the inhalation of α -amylase of mycogenic origin, and the number of cases studied with clinical and immunologic evaluation is few. Inhalation exposure to α -amylase is generally occupational (pharmaceutical and laboratory workers and bakers). However, the general population may be exposed by other routes, such as the ingestion of bread and bakery products containing α -amylase added during their processing.

We present the results of a study performed in a large group of pharmaceutical-industry workers exposed to α -amylase. The findings of the clinical questionnaire, skin and serum IgE tests, and inhalation and oral provocation tests have been described.

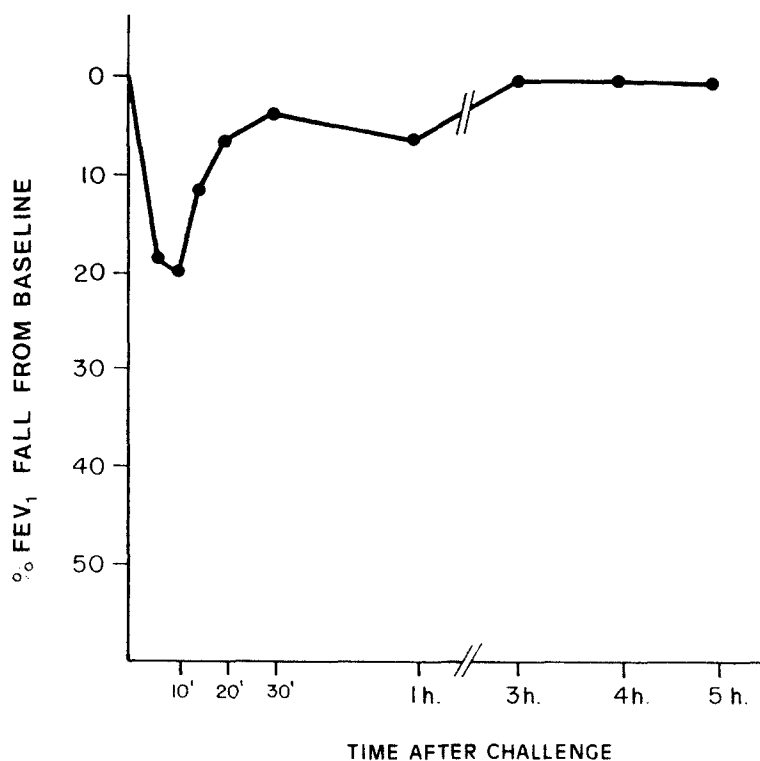


FIG. 3. Demonstration of an immediate asthmatic reaction after challenge with 0.5 mg of fungal α -amylase. *Abscissa* represents time after end of exposure.

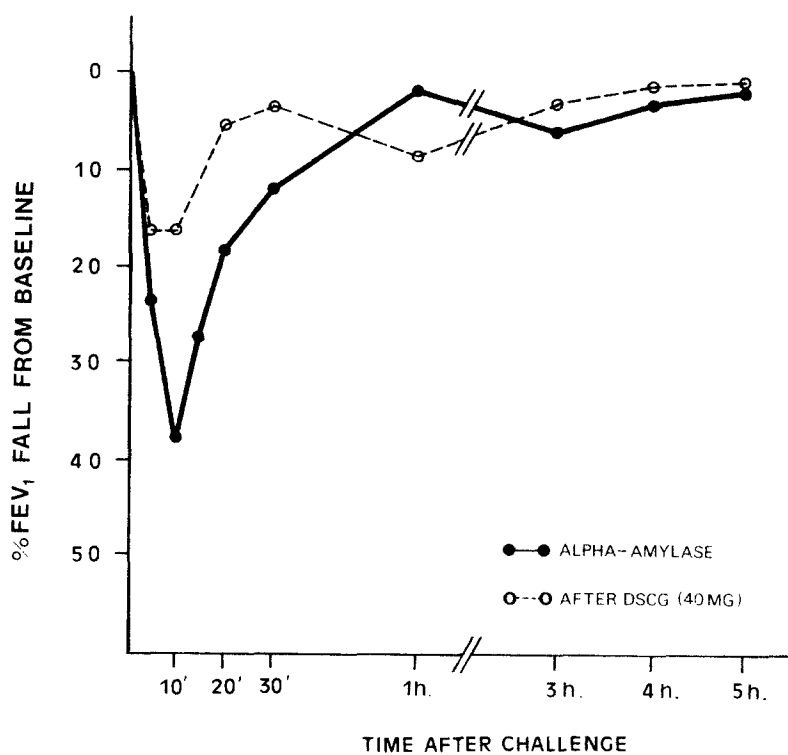


FIG. 4. Bronchial provocation test with α -amylase (●) and the same test performed 30 minutes after inhalation of 40 mg of disodium cromoglycate (○), which partially inhibited response.

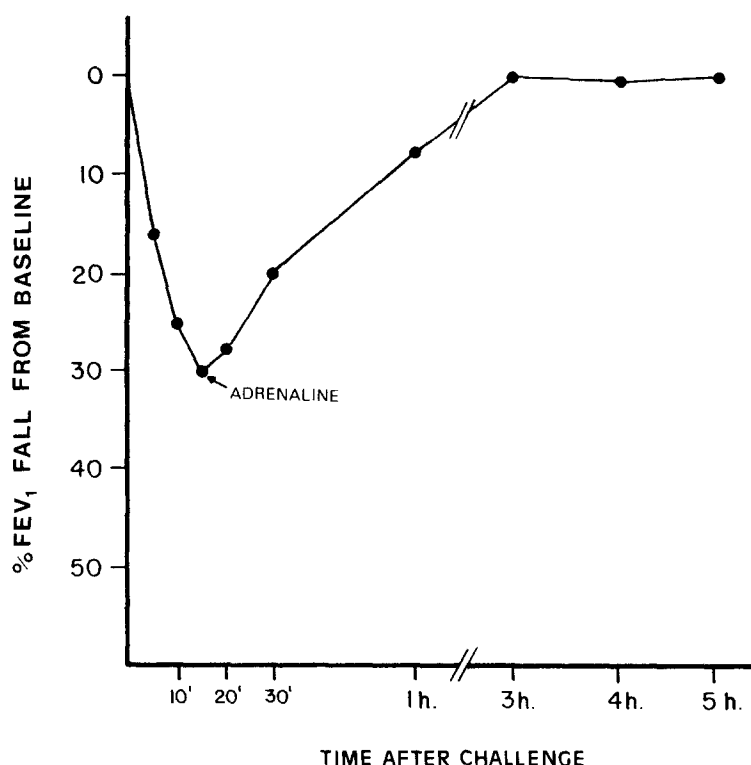


FIG. 5. Bronchial response after oral challenge with α -amylase (10 mg). *Abcissa* represents time after ingestion. Patient also had generalized urticaria at 15 minutes and was treated with subcutaneous adrenaline.

The level of exposure to α -amylase of the workers in the company surveyed has been classified as intense, intermediate, and low, depending on the activity of the worker in the factory and the frequency of exposure to this enzyme. Of the 26 patients with positive skin tests to α -amylase, 18 had an intense level of exposure, six had an intermediate level, and only two workers had a low level, thus suggesting the importance of this factor in the development of sensitization.

This study demonstrates that frequency of sensitization to α -amylase was high in the group studied (33.3%; 76.9% of the subjects were symptomatic). From these findings, it appears clear that an IgE-mediated immunologic mechanism was responsible for the symptoms presented by our patients. This fact was confirmed by the results of the skin and serum IgE tests. In some cases, controlled reexposure to α -amylase, either by bronchial or nasal provocation, induced the symptoms of rhinitis or BA with an immediate response.

In the six cases with positive bronchial challenges, only immediate response patterns were observed; there were no late or dual responses. These immediate responses, together with the positivity of the skin tests and the finding of specific IgE antibodies to α -amy-

lase, strongly suggest the existence of a specific IgE-dependent bronchoconstrictive mechanism.

In the bronchial reaction illustrated in Fig. 3, a very transient fall in FEV₁ with complete recovery at 30 minutes can be observed, suggesting the possibility that, in this case, the reaction might have been of an irritant type. However, this patient also demonstrated positive skin tests and IgE values to α -amylase, which is in agreement with the specificity of the reaction. Furthermore, the negativity of the bronchial challenges in 10 nonexposed workers with asthma and 10 asymptomatic exposed workers make unlikely the existence of irritant or pharmacologic mechanisms.

In contrast, six of the patients with positive skin tests presented with no clinical symptoms. These cases were probably of subclinical sensitization or latent allergy, but we do not know if these patients will develop allergic symptoms in the future.

Twenty-one of the 47 symptomatic workers were not sensitized to α -amylase but were possibly sensitized to other enzymes to which they were exposed (such as cellulase, papain, and lipase). These enzymes might well be responsible for their symptoms; otherwise, there was no etiologic relationship with the working environment.

We are unable to give any explanation of the im-

portance of specific IgG and its possible role in these workers, and further studies are necessary to clarify its significance. However, this presence might be interpreted as an evidence of environmental exposure to α -amylase and its immunogenic capacity.

An interesting finding is the result obtained in one patient with a positive oral α -amylase challenge. We have previously described the results of oral provocation with papain in five exposed sensitized workers who presented BA; on oral provocation, two of these patients presented a positive response with abdominal pain, vomiting, diarrhea, and urticaria but without respiratory symptoms.¹⁹ The symptoms in our present patient were intense, accompanied by a respiratory picture with wheezing and dyspnea that produced a 30% fall in FEV₁, requiring immediate treatment. However, this patient tolerated the ingestion of bread (that is supposed to contain fungal α -amylase) without any problem. This finding could be due to differences in the dose administered and/or heat denaturation of the enzyme during the baking process. To our knowledge, this is the first description in the literature of allergy to orally administered α -amylase. It is of clinical interest because of the oral use of α -amylase in pharmacotherapy and as an additive to flour contained in bread.

Unlike our findings in the case of cellulase sensitization,⁷ we could not demonstrate the existence of cross-reactivity between α -amylase and *A. oryzae*, the fungus that produces it. Moreover, the skin tests with *A. oryzae* were negative. We were also unable to demonstrate cross-reactivity between α -amylases of different origin (*A. oryzae* and pig pancreas).

From the previously mentioned results, we conclude that α -amylase behaves as a potent allergen capable of sensitizing a high number of exposed workers, giving rise to rhinitis and BA mediated by an allergic IgE-dependent mechanism. This enzyme should be recognized as an important causative agent of occupational asthma.

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