

Sensitivity to enzyme additives in laundry detergent workers

Raymond G. Slavin, M.D., and Charles R. Lewis, M.D.
St. Louis, Mo.

*An occupational disease of laundry detergent workers due to sensitivity to enzyme additives is described. Rhinitis and/or asthma was found in 66 workers and was presumably due to sensitivity to proteolytic enzyme products of *Bacillus subtilis*. A good correlation was found between clinical sensitivity to the enzyme preparation and (1) the presence of atopy and (2) immediate skin reactivity to the enzyme solution. The disease is thought to be representative of a type 1 immediate hypersensitivity and not a type 3 Arthus sensitivity. Environmental precautions in the plant resulted in a marked decrease in respiratory symptoms in the workers.*

In 1969, Flindt¹ and Pepys and associates² described allergic respiratory symptoms in English workers involved in the manufacture of enzyme-containing detergents. The symptoms of rhinitis and asthma were presumably due to enzyme products of *Bacillus subtilis* which are added to the detergents. The importance of the enzyme additives to the detergent industry is attested to by the fact that until recently approximately 75 per cent of all household detergents contained the enzyme additive. To investigate further the clinical and immunologic features of this occupational disease, 238 workers in a detergent factory were evaluated by careful history and by skin testing to a battery of inhalant allergens and a preparation of the *Bacillus subtilis* enzyme.

METHODS AND MATERIALS

Evaluation of workers: History and skin testing

A careful allergy history was taken in 238 workers in the detergent plant. Prick and/or intradermal skin testing was performed to a battery of common St. Louis allergens including mixed grasses, mixed trees, ragweed, and mixed molds.* If prick tests were negative, then intradermal tests were done. The *Bacillus subtilis* enzyme preparation was supplied by Lever Brothers as the lyophilized powder, Alcalase. Sterile buffered diluent* was added to make concentrations of 0.05, 0.5, and 5.0 mg. of protein per milliliter, and prick tests were performed with the solutions. Those workers considered atopic had either a personal history of allergy consisting of allergic rhinitis and/or extrinsic bronchial asthma or positive skin

From the Section of Allergy and Immunology, Department of Internal Medicine, St. Louis University School of Medicine, St. Louis, Mo. 63104

Supported in part by a grant from Lever Brothers, Inc., New York, N. Y.

Received for publication April 6, 1971.

Reprint requests to: R. G. Slavin, St. Louis University School of Medicine, Dept. of Internal Medicine, Section of Allergy and Immunology, 1325 South Grand Blvd., St. Louis, Mo. 63104.

*Hollister-Stier Laboratories, Downers Grove, Ill.

reactivity to at least one of the common allergens. Our criterion of positive skin reactivity was at least 10 mm. of erythema.

Passive transfer of skin sensitivity in man

Passive transfer of undiluted enzyme-sensitive serum to the skin of normal subjects was performed according to the classic Prausnitz-Küstner³ technique. Sensitized serum, heated to 56° C. for 2 hours, was also used.

Passive transfer of systemic sensitivity to the monkey

Macaca mulatta monkeys served as recipients of normal and allergic serum. On Day 1 the control monkey received 30 ml. of normal human serum intravenously and the experimental monkey 30 ml. intravenously of serum from a worker with respiratory symptoms due to the enzyme additive. On Day 2 the same amount of the same sera was again injected. On Day 3 both monkeys inhaled for 5 minutes an aerosolized 0.5 per cent aqueous solution of Alcalase in an ultrasonic tent.

Characterization of precipitins

Ordinary double gel diffusion⁴ was performed with workers' sera and normal control sera against the 5.0 mg. protein per milliliter of Alcalase solution. Sera were run through a Sephadex G-200 column, 5 cm. by 85 cm., with 1M acetate buffer, pH 4.1, and the major 7S and 19S fractions separated. Double gel diffusion was performed with the two fractions against the Alcalase solution. Fifty per cent ammonium sulfate was added to sera, and a solution of the resulting precipitate was placed in double gel diffusion against Alcalase.

Occupational health measures

Environmental precautions were introduced into the plant particularly in high hazard areas where workers were exposed to either premix or raw enzyme. These precautionary measures included the following: Many operations previously performed in the open were enclosed. Glove boxes were placed throughout the area. A number of procedures previously done by hand were now automated. In especially hazardous area, space suits with an air supply were provided. Disposable gloves and uniforms were discarded after one day's wear. Masks equipped with cartridge and filter were issued to employees and careful instruction given as to their use and importance. This was particularly emphasized to a group composed of machinists and electricians who had short but heavy exposure to the enzyme. Air monitoring was performed twice during each shift; collection time was 40 minutes and processing time one hour. The amount of enzyme accumulating in filters was determined by a ninhydrin dye test, read on a continuous flow spectrophotometer. If readings were above 5 glycine units, the enzyme operation was immediately shut down. Spot checks were performed on workers including interview, spirometry, and monitoring data. If any aspect of this check revealed a suspected abnormality, the worker was recalled in 2 or 3 days.

RESULTS

Clinical characteristics

Sixty-six workers had clear-cut symptoms associated with enzyme dust exposure. Five had rhinitis, 35 asthma, and 26 both rhinitis and asthma. The onset of symptoms of rhinitis or asthma varied from a few minutes to 6 to 8 hours following exposure. Affected workers had a variety of duties. Especially prominent were those who had brief but extreme exposure to enzyme dust. Pulmonary function tests on 40 acutely ill workers showed evidence of marked airway obstruction. Five of the most acutely involved workers were evaluated 2 weeks to 6 months after their acute episodes. Complete pulmonary function

TABLE I. The association of atopy with sensitivity to enzyme preparation

	Asymptomatic workers	Symptomatic workers
Nonatopic	118	6
Atopic	54	60
		$p < 0.001$

Of the 114 atopic workers, 85 had both positive allergy history and positive skin tests, 18 had negative allergy history and positive skin tests, and 11 had positive allergy history and negative skin tests.

TABLE II. The association of skin test reactivity* and clinical sensitivity to enzyme additives

Skin test	Asymptomatic workers	Symptomatic workers
Negative	114	10
Positive	58	56
		$p < 0.001$

*Prick tests.

testing, including carbon monoxide diffusion measurement, showed no abnormalities except for mild hyperinflation.

Table I indicates the relationship of the atopic state to symptoms due to enzyme sensitivity. Sixty of the 66 affected workers were classified as atopic. The chi square value is statistically significant at 62.8 ($p < 0.001$).

Table II indicates the association of symptoms due to the enzyme and skin reactivity to the Alcalase solution. Fifty-six of the 66 affected workers showed positive skin reactions (prick tests) to 0.05 to 0.50 mg. of protein per milliliter of solution. Fifty-eight of the 172 unaffected workers also showed skin reactivity, but the majority were positive only to 5.0 mg. per milliliter of solution. The chi square value of 53.9 is also statistically significant ($p < 0.001$).

Passive transfer of skin sensitivity to man

Immediate wheal and erythema reactivity to the Alcalase solution was successfully transferred from the sera of 3 positive workers to those of negative control subjects. The transfer of immediate skin reactivity was not successful with the heated sera.

Passive transfer of systemic sensitivity to the monkey

On Day 3, the control monkey who received two injections of normal human serum appeared perfectly normal after Alcalase aerosolization. In contrast, the experimental monkey who received serum from an enzyme-sensitive worker developed marked cyanosis, restlessness, and dyspnea after several minutes of aerosolization. Respirations were labored and tachypnea of 40 per minute was present.

Characterization of precipitins

All workers' sera as well as normal control sera showed precipitation bands against the Alcalase solution. After separation of the sera on a Sephadex G-200 column, the precipitating factor was found in the 19S fraction. A solution of

the precipitate formed after addition of 50 per cent ammonium sulfate to serum showed no precipitin bands against Alcalase.

Occupational health measures

With the institution of the preventive medicine measures discussed, the prevalence of respiratory symptoms in the detergent workers has declined markedly. Dispensary visits for respiratory symptoms dropped from 45 in the 3 months preceding institution of preventive medicine measures to 10 in the 3 months following. In addition, the severity of the symptoms noticeably decreased.

DISCUSSION

It seems evident that sensitivity to the enzyme preparation of *Bacillus subtilis* is the basis for a true occupational disease in laundry detergent workers. Sensitive workers have a type 1 immediate-type hypersensitivity. Eosinophilia of peripheral blood is not present, and chest roentgenograms are normal. Other reports⁵ have suggested residual impairment of pulmonary function, including diffusion defects, in workers experiencing dyspnea and bronchospasm following enzyme dust exposure. In this study, pulmonary function tests, including carbon monoxide diffusion measurements, failed to show abnormalities except for mild hyperinflation. Sera from both sensitized workers and normal control subjects demonstrated precipitin bands to the Alcalase solution. The precipitating factor was found in the 19S peak after Sephadex G-200 separation. The gamma globulin fraction obtained by ammonium sulfate precipitation showed no precipitin bands against the enzyme solution. We would agree with others^{6, 7} that this represents nonspecific precipitation on a nonimmunologic basis, probably by α_2 -macroglobulins, and is not indicative of a type 3 Arthus sensitivity.

Our study is in agreement with that of Newhouse and associates⁸ in that an atopic background seems to predispose the worker to enzyme sensitivity, and a good correlation was seen between symptoms and immediate skin reactivity to the enzyme. This would suggest that atopic workers should not be exposed to industrial levels of the enzyme dust and that, with development of immediate skin reactivity to the enzyme, the workers should be removed from further exposure or, at the very least, observed carefully for the development of symptoms.

The occupational health and preventive medicine aspects of this occupational disease have been particularly interesting to observe. An acute episode involving a number of workers was traced to a block in the ventilation system due to the freezing of an opening on the plant roof. The institution of appropriate preventive medicine measures resulted in a dramatic decrease in respiratory symptoms.

This study would not have been possible without the cooperation of the management and workers of Lever Brothers, Inc. We would particularly like to acknowledge the aid and advice of Dr. David Justice, Dr. Edward J. Singer, and Mr. Dennis Martin.

REFERENCES

- 1 Flindt, M. L. H.: Pulmonary disease due to inhalation of derivatives of *Bacillus subtilis* containing proteolytic enzyme, *Lancet* 1: 1177, 1969.
- 2 Pepys, J., Hargreave, F. E., Longbottom, J. L., and Faux, J.: Allergic reactions of the lungs to enzymes of *Bacillus subtilis*, *Lancet* 1: 1181, 1969.
- 3 Prausnitz, C., and Küstner, H.: Studien über die Ueberempfindlichkeit, *Zentralbl. Bakteriol. (Naturwiss)* 86: 160, 1921.
- 4 Ouchterlony, O.: Diffusion-in-gel methods for immunological analyses, *Progr. Allerg.* 5: 1, 1958.
- 5 Franz, J., McMurrian, K. D., Brooks, S., and Bernstein, I. L.: Clinical, immunologic, and physiologic observations in factory workers exposed to *B. subtilis* enzyme dust, *J. ALLERGY* 47: 170, 1971.
- 6 Monte, V., and Dolovich, J.: Binding of the allergenic *Bacillus subtilis* proteinases with α_2 -macroglobulin, *Excerpta Medica Int. Congress Series* No. 211, 1970, p. 14. (Abst.)
- 7 Belin, L., Falsen, E., Hoborn, J., and André, J.: Enzyme sensitization in consumers of enzyme-containing washing powder, *Lancet* 2: 1153, 1970.
- 8 Newhouse, M. L., Tagg, B., Pocock, S. J., and McEwan, A. C.: An epidemiological study of workers producing enzyme washing powders, *Lancet* 1: 689, 1970.