

## Diagnostic tests in the skin and serum of workers sensitized to *Bacillus subtilis* enzymes<sup>\*†‡</sup>

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### Summary

Two allergen pools of commercial detergent enzymes were prepared as skin test reagents: (1) Carlsberg type, composed of three products containing subtilopeptidase A, and (2) BPN type, composed of two products containing subtilopeptidase B and  $\alpha$ -amylase.

In 100 non-exposed controls a reaction suggesting primary irritancy was found at protein concentrations greater than 1  $\mu$ g/ml intradermally or 1 mg/ml by prick test. Intradermally at 10  $\mu$ g/ml weals were accompanied by less pronounced flare reactions than observed in specifically sensitized enzyme workers. At 100  $\mu$ g/ml the reactions were like strong specific reactions. False positive prick test reactions occurred irregularly at 10 mg/ml. In 100 sensitized enzyme workers, reactions were elicited at concentrations from 1.0 to  $10^{-5}$   $\mu$ g/ml intradermally and from 1000 to 1  $\mu$ g by prick test. Intradermal and prick tests correlated well ( $r_s = 0.84$ ,  $P < 0.001$ ). Ratings of symptom severity upon exposure obtained from questionnaires were significantly correlated with skin test reactivity ( $P < 0.01$ ). RAST performed on sera collected simultaneously also correlated significantly with symptom scores. PCA tests in monkeys were less sensitive.

Standardized test reagents allow diagnostic skin testing by either intradermal or prick test in *B. subtilis* enzyme sensitive patients. A clear distinction between primary irritant reactions and true sensitization was made on the basis of the concentration required to elicit a reaction.

### Introduction

In the U.S.A. proteolytic enzymes from *B. subtilis* were first added to detergents in

\* Supported by a contract with The Soap and Detergent Association, New York City.

† The protocol for these studies was developed by a subcommittee of the Enzyme Committee of The American Academy of Allergy. The subcommittee consisted of Merrill W. Chase, Lawrence M. Lichtenstein, Bernard Levine, David A. Levy and Philip S. Norman.

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1967. Three years later, 70–75% of all detergents sold contained enzymes (Carter & McMurray, 1970). These products had been introduced on the market a few years earlier in Europe and the first report on sensitization to enzymes came in 1969 from England where Flindt described twenty cases of sensitivity among exposed workers in a detergent factory (Flindt, 1969). In these cases positive immediate prick test reaction to enzyme preparations supported the diagnosis of allergy.

Later epidemiologic surveys in England, as well as the U.S.A. and Canada, have clearly shown that *B. subtilis* enzymes are allergenic when inhaled as a dust and, depending upon the degree of exposure, workers handling these products have become sensitized in a frequency of 20–60% (Newhouse *et al.*, 1970; Greenberg, Milne & Watt, 1970; Weill, Waddell & Ziskind, 1971; Slavin & Lewis, 1971; Shapiro & Eisenberg, 1971; Little & Dolovich, 1973). The pulmonary reactions seen in some workers were suspected by Pepys principally as immunological type III reactions, with the addition of type I immediate responses giving asthma (Pepys *et al.*, 1969).

Although Pepys' conclusion has not been supported by other researchers, the pathogenesis of the disease has not been entirely clear (Editorial, 1971). There seems, however, to be a general agreement that a type I IgE-mediated hypersensitivity mechanism dominates in most cases of enzyme allergy and the correlation between symptoms and positive immediate skin tests has been high in workers (Newhouse *et al.*, 1970; Greenberg, Milne & Watt, 1970; Weill, Waddell & Ziskind, 1971; Slavin & Lewis, 1971; Shapiro & Eisenberg, 1971; Little & Dolovich, 1973).

However, the interpretation of skin test reactions is made difficult by the primary irritant effect of the active enzyme and other substances at higher concentrations. Furthermore, test solutions of enzyme material may contain measurable amounts of histamine. The multiplicity of test antigens used, their frequent lack of similarity to the product to which workers are exposed, variations in testing techniques, and varying criteria for reading skin reactions preclude useful quantitative comparisons of skin testing results reported by the various investigators.

Decreased levels of enzyme dust in the factory environment, as a result of dust control measures, seem to have diminished the frequency of *B. subtilis* enzyme sensitization to a large extent (Weill *et al.*, 1971; Little & Dolovich, 1973), although the precise risk of low level exposure in the future for both workers and consumers is not known (Belin *et al.*, 1970; Bernstein, 1972). Standardized diagnostic reagents and tests would facilitate follow-up studies of such hazards in the future and serve the purpose of guiding the rapidly developing enzyme technology in avoiding them. This work will demonstrate how such diagnostic tests correlate with each other and how they relate to sensitivity to the enzymes which have been used in enzyme detergents in the U.S.A.

### Materials

The following five commercial grade *B. subtilis* enzymes were obtained from the Research and Development Department of the Procter & Gamble Company, Ivorydale Technical Center, Cincinnati, where they had been collected from the manufacturers: (a) Pfizer Maxatase (880,000 D.U.); (b) Novo Alcalase (4 Anson Unit); (c) Wallerstein 201; (d) Monsanto DA-10, and (e) Milezyme 8X. There was no information on these labels about the production or lot number of the manufacturer. The bottles were stored tightly sealed under refrigeration during the time of the study.

Separate 10% solutions were made of dry enzyme powder, allowing it to dissolve as completely as possible during mild stirring in sodium acetate buffer, pH 5.0.

The protein of the sample, diluted appropriately, was precipitated with 10% trichloroacetic acid and pelleted by centrifugation at 1,500 rev/min for 5 min. After decanting of the supernatant the precipitated protein was redissolved in 1 mol/l sodium hydroxide, and determined by the method of Lowry *et al.* (1951). A 00 Lysozyme 3X crystallized, Sigma No. L-6870, was used as a standard.

#### *Allergen test pools*

Enzyme preparations a, b and c, which were of subtilisin A or Carlsberg type contained 10, 12 and 19% protein, respectively, whereas d and e were of B type and contained 21 and 19% protein. Subtilisin A enzymes were combined to make test pool 1 and subtilisin B enzymes to make pool 2. The individual enzymes were added in amounts which gave each enzyme an equal proportion of the protein in each pool and a total protein concentration of 10 mg per ml.

As an additional skin test reagent, a dialysed preparation of Alcalase® Novo (enzyme b) was also used in eighty-six of the workers studied. This preparation, designated Antigen A, was recommended by How & Cambridge in 1971 as an appropriate prick test and RAST reagents for use in the detection of allergic reactivity to enzymes in washing products.

#### *Test subjects*

(A) *Allergic individuals.* The three soap and detergent companies which participated in this study referred 117 employees earlier diagnosed as, or suspected to be, allergic to *B. subtilis* enzymes by the investigator during the spring of 1973. All three companies started to produce enzyme-containing detergents in 1968–1969. Two years later Procter & Gamble and Colgate markedly reduced their production of these products while Lever Bros completely stopped making them.

According to the production schedules, the sixty-one workers from the Procter & Gamble Company (fifty-one in the Baltimore plant and ten at Ivorydale Technical Center, Cincinnati) had been exposed to both the pool 1 and pool 2 enzymes, while the twenty-seven employees from Colgate (New Jersey plant) and the twenty-nine from Lever Bros (Edgewater, N.J. and Baltimore plants) had been mainly exposed to the pool 1 enzymes and to a minor extent to pool 2 enzymes. All tested workers participated on a voluntary basis after signing an approved consent form.

(B) *Non-sensitized controls.* Two groups of fifty volunteers served as non-sensitized controls. Fifty volunteers in Baltimore comprised thirty women and twenty men between 19 and 46 (mean 30) years of age and they were tested at The Good Samaritan Hospital. The other fifty were exclusively men between 21 and 48 (mean 27) living in Cincinnati where the testing was organized by Dr Leonard Bernstein at the Cincinnati General Hospital. A personal or family history of allergy was noted in 38% of the Baltimore and in 48% of the Cincinnati group. None had experienced any allergic symptoms suggesting hypersensitivity to detergent enzymes.

#### *Clinical history*

A clinical history was obtained from all test subjects through questionnaires. The worker's questionnaire included questions concerning industrial exposure and was completed by an interviewer, whereas the control subjects completed their own

questionnaires which included questions concerning the use of laundry products. In order to compare the clinical symptoms of enzyme allergy with the results of the skin and serological tests, symptom severity was scored from information obtained by the questionnaires filled out by the workers. The difficulty in evaluating symptoms experienced 3-5 years earlier, in addition to the impossibility of grading the degree of allergen exposure in workers who often changed working areas in the plants, resulted in the choice of a simple scoring system. The worker was asked about work-connected symptoms which started after the introduction of *B. subtilis* enzymes in the plants. Three questions referred to eyes (itching, redness, discharge), three to the nose (sneezing, discharge, stuffy nose), four to the chest (cough, wheezing, tightness, soreness) and four to general symptoms (fatigue, fever, chills, joint pains). The general symptom questions were asked only if questions about respiratory symptoms had been answered affirmatively. Being of a more serious nature, each chest symptom was given 2 points; all others 1 each. Thus, a maximum score of 18 could be reached.

#### *Skin test procedures*

(A) *Test areas and sites.* All skin tests were made in duplicate at sites paired diagonally on either side of the arbitrary midpoint of the volar surface of the forearms within an area 3 cm distal to the cubital fold and 3 cm proximal to the wrist. One arm was used for the prick test and the other for the intradermal test. Six paired sites were marked on the test area using marker pens. The duplicate test sites were always distal and contralateral from the midpoint in relation to the original sites. Within this given pattern the intradermal and prick tests were randomized.

(B) *Preparation of dilutions.* After rehydration of the lyophilized stock solutions the enzyme allergen pools 1 and 2 were further diluted in ten-fold dilution steps using a phosphate-buffered saline of pH 6.4, since the sodium acetate buffer in which the enzymes were primarily dissolved had an uncomfortable stinging effect when injected into seven volunteers. Preliminary studies also had shown that activation of the alkaline peptidases was decreased at this pH of the phosphate buffer system. The diluent had the following composition:  $\text{KH}_2\text{PO}_4$  860 mg (0.086%),  $\text{Na}_2\text{HPO}_4$  49 mg (0.0049%), NaCl 5000 mg (0.5%), phenol 4000 mg (0.4%), distilled water q.s. 1.000 ml.

(C) *Intradermal technique.* Becton, Dickinson and Company, Rutherford, New York, sterile, disposable 1 ml tuberculin syringes and disposable Yale 26 ga.  $\times \frac{3}{8}$  in. needles were used for the intradermal tests. The skin was washed with isopropyl alcohol and marked for testing. The needle was introduced into the epidermis with the bevel face down to the skin surface and a volume of 0.02 ml was injected. It was found feasible and accurate to inject the test extract under careful inspection of the test site. The intradermal injection of 0.02 ml raised an immediate weal with a diameter of 3 mm. When this volume of a 0.01% histamine solution (0.275 mg histamine phosphate, Eli Lilly & Company, Indianapolis, Indiana, per ml of diluent) was injected intradermally in forty-two of the control subjects, the weal reaction after 15 min had a mean diameter of 11.5 mm with a standard deviation of 2.1. All intradermal tests in this study were made by the investigator.

(D) *Prick test technique.* The prick test solutions were applied as single drops from plastic drop vials at pre-marked skin test sites. A disposable sterile 26 ga.  $\times \frac{1}{2}$  in. needle (Becton, Dickinson and Company) was passed through the drop and inserted into the skin in a position almost parallel to the skin with the bevel directed away from

the surface. With a slight lifting of the skin, the needle was then withdrawn and the solution was wiped off approximately 1 min later with a paper tissue. The prick lesion was made superficially enough in most cases not to cause any bleeding. The original and the duplicate tests were pricked with the same needle. Separate needles were used for each concentration and pool. With this prick test technique a 1% solution of histamine after 15 min gave a weal reaction 6.4 mm mean diameter in seven control subjects, the standard deviation being 2.1 mm.

(E) *Recording of skin reactions.* The skin reactions were read after 15 min by the investigator using a transparent plastic ruler with a millimetre scale (Reaction Guide, Center Laboratories, Inc.). The longest and the shortest diameter of both weal and flare were measured and recorded on the skin test data charts. Mean diameters are presented in the data which follows.

(F) *Titration of skin sensitivity on allergic workers.* Titration of the skin sensitivities started with prick tests at the lowest concentration of the prick test series, usually 10 µg protein per ml. Selection of appropriate concentrations for the first set of intradermal tests was guided by the results of the prick tests. The tests were continued at 15 min intervals with increasing concentrations until a clear cut positive reaction was noted with a weal diameter at least 5 mm larger than the diluent control after intradermal testing and at least 3 mm larger than the same control after prick testing, and in both instances a flare extending beyond the margin of the weal.

#### *Serological tests*

A volume of 20–30 ml of blood was drawn from each test subject into vacutainer tubes before the skin testing. The separated serum was divided into three or four portions and stored at –20°C. until analysed. As antigens, the same test pools as used in the skin tests were employed.

(A) *The radioallergosorbent technique (RAST)* was performed on sera from all test subjects by Dr R. Verbruggen and H. Krauss at the Ivorydale Technical Center of the Procter & Gamble Company, Cincinnati, Ohio. The test pools were coupled to Sephadex particles as originally described by Wide, Bennich & Johansson (1967). A sheep anti-IgE serum supplied by Dr Gerald J. Gleich was used after labelling with <sup>125</sup>I. Before incubation with the test serum, the enzyme coupled particles were treated with an enzyme inactivator, diisopropyl fluoro-phosphate (DIFP). This procedure was found to increase the sensitivity of the test system significantly, particularly when pool 1 was used. An uptake on the particles of more than 3% of the positive standard serum (after subtraction of the uptake from a negative standard serum run at the same time) was counted as a positive result.

RAST determinations were also kindly performed on fifty-three sera from sensitized subjects by Pharmacia AB, Uppsala, Sweden, in the Phadebas® modification in which the test antigens were coupled to paper discs. This method did not include an enzyme inactivation step. The results were graded according to the Phadebas® system into classes from 0 to 4, where class 0 is considered to be a negative response and classes 1–4 indicate the presence of circulating specific IgE in increasing amounts. This grading is based upon a positive reference serum with high IgE titre to birch pollen allergen and the classes correspond to the radioactive uptake on standard birch pollen allergen discs tested with undiluted serum and serum diluted 1:5, 1:25 and 1:50.

(B) *Passive cutaneous anaphylaxis (PCA) in monkeys* were conducted on: (a) all

sera at Hazleton Labs, Falls Church, Virginia, and (b) on selected sera at the Division of Clinical Immunology, Johns Hopkins University School of Medicine, Baltimore, Maryland. All these skin tests were made on the abdomen and thorax of Sernylan (Parke, Davis Co.) tranquilized *Cynomolgous* monkeys using a 24 hr sensitization time. The tests used 250  $\mu$ g protein of each pool for challenge and 2 ml of 2% Evans blue saline solution was given together with the antigen for visualization of the reactions. The (a) test results were expressed as the width  $\times$  length of the blueing reactions read at 30 min. Sera subjected to the (b) test were titrated with five-fold dilutions and the results expressed as + when only undiluted serum gave a reaction and as one additional + for each dilution step at which a blueing reaction could still be seen. These were read 45 min after the challenge with 1 mg protein of the test pool together with 2 ml of a 1% Evans blue in saline.

## Results

### (A) Control subjects—'non-allergic' individuals

As the Cincinnati and the Baltimore control groups differed not only in their environment but also in their age and sex composition, the results of the skin tests are given separately in Table 1. No significant differences between the skin test reactions in the two groups were noted. The individual weal responses to the highest concentration of the pools were also remarkably uniform as indicated by the small standard deviations (0.9–1.3 mm) from the mean diameter values. The strong weal reactions elicited by both pools at this concentration (100  $\mu$ g protein per ml) were surrounded by distinct flare reactions, making such skin responses indistinguishable from specific intradermal reactions. At 10  $\mu$ g per ml smaller but definite weals were also noted in most cases; but only in 10 and 37% of the reactions elicited by pool 1 and pool 2 respectively, were they accompanied by flares extending well beyond the margin of the weals. Both control groups showed a slightly higher reactivity to pool 2 than to pool 1.

**Table 1.** Mean weal (W) and flare diameters (F) in mm elicited by intradermal injection of 0.02 ml enzyme allergen pools 1 and 2 at three concentrations in each of 50 non-sensitized individuals from Cincinnati and Baltimore (s.d. in parentheses)

|        |   | Diameter of reaction (mm) at |               |                |                     |
|--------|---|------------------------------|---------------|----------------|---------------------|
|        |   | 1 $\mu$ g/ml                 | 10 $\mu$ g/ml | 100 $\mu$ g/ml |                     |
| Pool 1 | W | 2.0 (2.3)                    | 5.0 (1.7)     | 7.8 (1.3)      | Cincinnati controls |
|        | F | Not calculated               | 2.0 (5.0)     | 15.4 (8.4)     |                     |
| Pool 2 | W | 3.1 (2.5)                    | 6.3 (1.1)     | 9.0 (0.9)      |                     |
|        | F | Not calculated               | 4.7 (5.7)     | 19.2 (7.7)     |                     |
| Pool 1 | W | 1.4 (1.8)                    | 4.3 (1.7)     | 7.7 (1.2)      | Baltimore controls  |
|        | F | Not calculated               | 1.9 (5.1)     | 15.6 (7.1)     |                     |
| Pool 2 | W | 1.6 (1.8)                    | 6.1 (1.2)     | 9.0 (1.2)      |                     |
|        | F | Not calculated               | 8.1 (7.3)     | 18.3 (7.0)     |                     |

**Table 2.** The frequency of weal reactions noted among 200 prick tests in 100 'non-allergic' controls using three concentrations of enzyme allergen pools 1 and 2. Figures indicate number of weal reactions by each pool at each concentration grouped according to weal size

| Weal diameter (mm) | Number of test observations |        |         |        |           |        |
|--------------------|-----------------------------|--------|---------|--------|-----------|--------|
|                    | 10 mg/ml                    |        | 1 mg/ml |        | 100 µg/ml |        |
|                    | Pool 1                      | Pool 2 | Pool 1  | Pool 2 | Pool 1    | Pool 2 |
| 0                  | 109                         | 99     | 174     | 154    | 194       | 196    |
| 1                  | 22                          | 20     | 14      | 20     | 2         | 3      |
| 2                  | 41                          | 47     | 10      | 22     | 0         | 0      |
| 3                  | 20                          | 21     | 1       | 2      | 3         | 1      |
| 4                  | 5                           | 9      | 0       | 2      | 1         | 0      |
| 5                  | 2                           | 3      | 1       | 0      | 0         | 0      |
| 6                  | 1                           | 0      | 0       | 0      | 0         | 0      |
| 7                  | 0                           | 1      | 0       | 0      | 0         | 0      |

At a concentration of 1 µg protein per ml, no reactions to any of the pools were recorded in the two control groups.

Weal reactions to the prick tests in the controls were seen much less frequently, although the number and size of reactions increased considerably when the highest test concentration (10 mg per ml) was reached as seen in Table 2. Weal reactions at this concentration were, however, irregular, both with regard to frequency and reproducibility. Out of the 200 prick tests with each pool, pool 1 gave twenty-seven and pool 2 gave thirty-three reactions with weal diameters of 3 mm or more. Only four out of the former and eight out of the latter were reproduced in the same test subject at each of the paired test sites.

#### (B) Allergic subjects—workers in detergent plants

(1) *Prick and intradermal tests.* Titration of the intradermal and prick test sensitivities to the enzyme pools in the allergic subjects were made on seven different

**Table 3.** Mean weal diameters of positive intradermal skin reactions determined at different test occasions in *B. subtilis* sensitive workers from three detergent companies. Number of test subjects in parentheses

|                       | Intradermal tests |                | Prick tests    |                |
|-----------------------|-------------------|----------------|----------------|----------------|
|                       | Pool 1            | Pool 2         | Pool 1         | Pool 2         |
| Colgate-Palmolive     | 6.7 ± 1.7 (23)    | 7.0 ± 1.3 (24) | 3.8 ± 1.2 (23) | 3.9 ± 1.1 (24) |
| Procter & Gamble (a)* | 7.1 ± 1.9 (38)    | 8.2 ± 1.9 (47) | 4.1 ± 1.3 (41) | 4.7 ± 1.5 (35) |
| (b)                   | 6.9 ± 1.2 (10)    | 7.2 ± 0.9 (10) | 4.5 ± 1.1 (10) | 4.5 ± 1.4 (10) |
| Lever Bros            | 6.9 ± 0.8 (25)    | 7.1 ± 1.2 (25) | 4.4 ± 1.0 (21) | 4.2 ± 1.2 (20) |

\* (a) Baltimore plant; (b) Cincinnati plant.

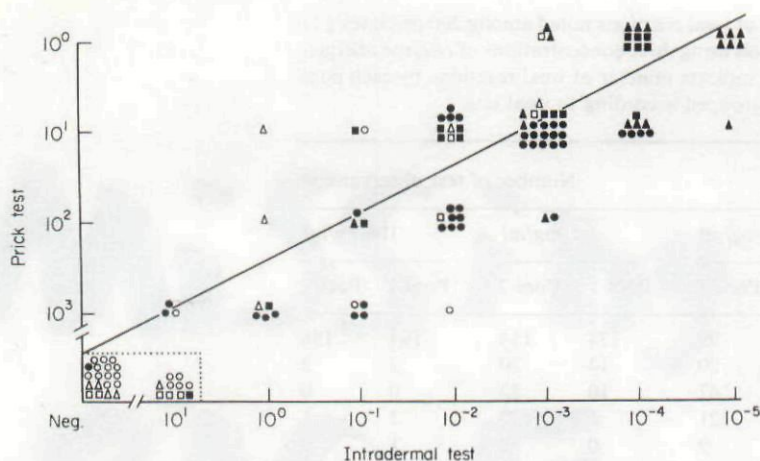


Fig. 1. Skin sensitivities to pool 1 in 117 presumably sensitized workers from the soap and detergent industry as titrated by the prick and intradermal test method. Workers from Procter & Gamble =  $\circ$ , C & P =  $\triangle$  and Lever =  $\square$ . Those indicated by closed signs were RAST positive.  $r = 0.84$ ;  $P < 0.001$ .

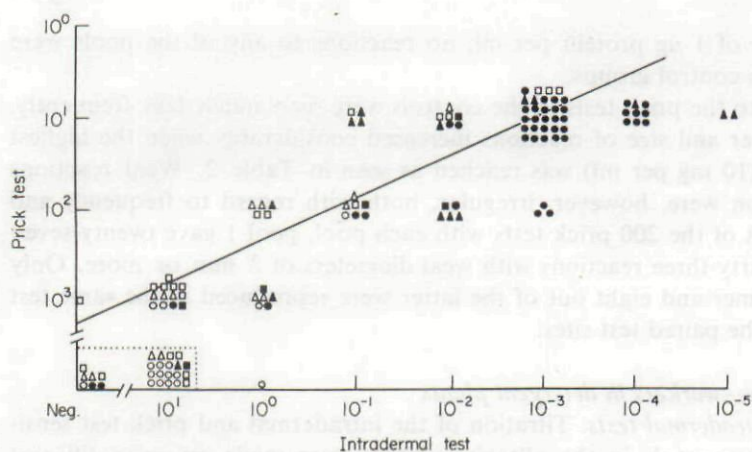


Fig. 2. Skin sensitivities to pool 2 in 117 presumably sensitized workers from the soap and detergent industry as titrated by the prick and intradermal test method. Subjects represented by closed signs were RAST positive.  $r = 0.85$ ;  $P < 0.001$ .

occasions during the spring of 1973. In order to show the uniformity of the determinations of the skin responses obtained at various times, the mean weal diameters of the positive threshold reactions which were taken to indicate the skin sensitivities are given in Table 3. These mean diameters were only slightly and insignificantly different, intradermal weals varying between 6.7 and 8.2 mm and prick test weals between 3.8 and 4.7. The mean difference between the weal diameters of the paired test reactions was  $0.7 \text{ mm} \pm 0.5$  for intradermal and  $1.1 \text{ mm} \pm 1.1$  for prick tests, the more distally located reactions regularly being smaller than the ones closer to the cubital fold on the volar surface of the forearm.

The threshold reactions were elicited over a concentration range of  $10$ – $10^{-5} \mu\text{g}$  per ml intradermally and  $1,000$ – $1 \mu\text{g}$  per ml by prick test. By both methods the

Colgate & Palmolive (C & P) and Lever workers reacted more strongly to pool 1 than to pool 2. The results of the prick tests and the intradermal tests correlated well with a correlation coefficient of 0.84 ( $P < 0.001$ ) with pool 1 (Fig. 1) and a correlation coefficient of 0.85 ( $P < 0.001$ ) with pool 2 (Fig. 2). In most instances the concentration of test pool required to elicit a positive prick test reaction was 1,000 to 10,000 times higher than that required to obtain an intradermal response. In a few subjects, however, as little as 100 or as much as 100,000 times higher concentration was needed for a prick test response. With pool 1 negative prick test reactions were found at the highest test concentration (1 mg protein per ml) in twenty-nine of the 117 supposedly sensitized workers. Eleven of these workers had positive intradermal reactions at 10  $\mu$ g per ml while the others were negative. With pool 2, twenty-six of the 117 were prick test negative at 1 mg per ml and nineteen of these were positive intradermally at 10  $\mu$ g per ml. These intradermal reactions were considered as non-specific in nature since reactions indicative of primary irritation had been found to a similar extent at this concentration in the non-allergic control group. This conclusion was supported by the RAST determinations which were generally negative in these workers.

It should be noted that some individuals gave a positive reaction to intradermal tests at a level considered non-specific (10  $\mu$ g per ml) but gave positive prick test reactions at a concentration considered specific (1 mg per ml). With pool 1 this happened in three individuals and with pool 2 in thirteen individuals. The converse, i.e. positive at 1  $\mu$ g per ml intradermally and negative by prick test at 1 mg per ml, occurred in one individual with pool 2. It appears, with pool 2 at least, that the prick test disclosed some specific positive reactions in individuals whose intracutaneous test was positive only at a level which could not be clearly identified as specific. In ten of thirteen such instances, however, the workers were exposed to, and more sensitive to, pool 1 enzymes.

(2) *Comparative prick test with AgA.* The English prick test reagent, antigen A, was as diagnostic as pool 1 in all the fifty-six C & P and Lever workers who were always equally or more sensitive to pool 1 than pool 2.

In nine of forty skin-sensitive Procter & Gamble workers (30%), the outcome of prick testing with AgA was, however, negative. These American workers were predominantly sensitive to pool 2, and a large proportion (60%) of those who were AgA negative also had a very low level of prick test reactivity to pool 1.

(3) *RAST determination and correlation with skin sensitivity.* The sera of thirty-three Procter & Gamble and twenty-one C & P employees were subjected to RAST determination by the laboratories of both Procter & Gamble and Pharmacia AB, Uppsala, Sweden. As to the qualitative results, there was 93% agreement using pool 1 and 89% agreement using pool 2. A Spearman rank-order analysis of the titres of the determination judged as positive by both laboratories showed close correlation. The correlation coefficient was 0.91 with pool 1 and 0.71 with pool 2 ( $P < 0.001$ ).

Assuming specific skin sensitivity to be disclosed by a positive intradermal test with 1  $\mu$ g protein per ml or less and a positive prick test with 1 mg protein per ml or less, 83 and 88% of the RAST values of Procter & Gamble (117 tests) and Pharmacia (fifty-three tests) respectively were in agreement with the skin reactivity. The Procter & Gamble RAST disagreement (17%) was due to an equal number of the two combinations: negative skin test/positive RAST and positive skin test/negative RAST, while the reason for Pharmacia RAST disagreement (12%) was with one exception the combination: negative RAST/positive skin test.

Thus, the Pharmacia laboratory—with its larger experience—performed RAST determinations with high specificity, but with a sensitivity below that of the skin tests (prick and intradermal tests exhibiting an equal sensitivity). In comparison, the Procter & Gamble determinations were slightly less sensitive and less specific. As a consequence of the lower specificity of the Procter & Gamble RAST determinations about 10% of the 100 control subjects were reported as positive. This result was not correlated with any clinical or skin test signs of *B. subtilis* enzyme sensitivity. It was, however, apparent that most of these positive determination were made at particular occasions, indicating experimental difficulties with the reproducibility of the assays.

(4) *Monkey PCA tests in relation to RAST.* Monkey PCA results were furnished by the Procter & Gamble laboratory on the sera of Procter & Gamble workers. As a general finding, positive monkey PCA reactions were elicited by sera with high RAST values (Procter & Gamble Laboratory determinations) but were not produced by more than half of the sera with lower but still positive RAST.

The monkey PCA results of the Procter & Gamble Laboratory were in good agreement with those obtained on sera also analysed at the Division of Clinical Immunology, Johns Hopkins University School of Medicine.

A total of forty-three sera were subjected to monkey PCA titrations by this latter laboratory and to RAST determination by the Pharmacia Laboratory. The results are plotted in Fig. 3. They confirmed the results furnished by Procter & Gamble Laboratory with these test methods. In addition they show a poor correlation between the monkey PCA titre and the RAST value when both parameters were positive.

Since the monkey PCA test turned out to be much less sensitive in detecting enzyme specific, circulating IgE antibodies than the RAST method, no further sera were subjected to this test.

(5) *Clinical symptoms in relation to skin sensitivity and RAST.* About 75% of the symptomatic workers gave an affirmative answer to the question about chest symptoms in connection with exposure to detergent enzymes. Most of them had also experienced allergic symptoms in the eyes and/or nose. In somewhat less than 25%, symptoms in

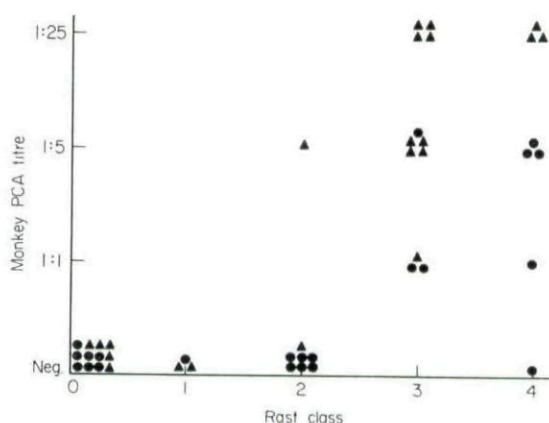


Fig. 3. Comparison of monkey PCA titre and RAST class. Triangles indicate workers mainly sensitized and challenged with enzyme pool 1 while circles represent sera of workers sensitized and challenged with enzyme pool 2.

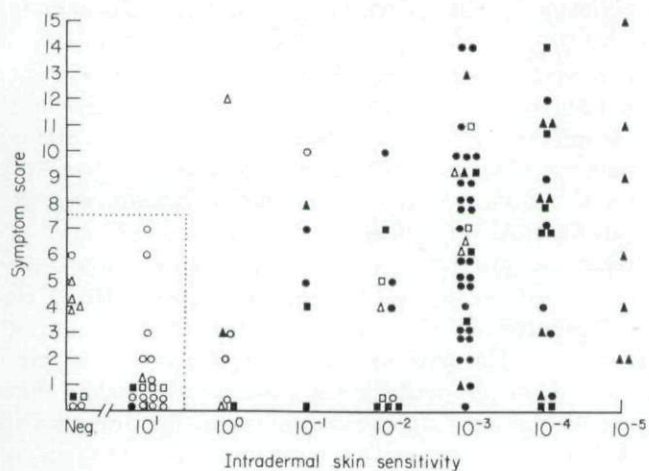


Fig. 4. Relationship between symptoms of *B. subtilis* enzyme allergy and intradermal skin sensitivity to the most reactive allergen test pool in 117 presumably sensitized workers from the soap and detergent industry.  $r_s = 0.4$ ;  $P < 0.01$ .

the upper respiratory tract were the only clinical sign of their allergy. Only 7% of the symptomatic workers also reported such general symptoms as fever and chills.

Symptom severity was compared with the level of intradermal skin sensitivity to pool 2 in the Procter & Gamble group and with the skin sensitivity to pool 1 in the C & P and Lever groups, since these were the pools to which the respective group was most sensitive. Spearman rank-order correlation analyses gave  $r_s$  values between 0.41 and 0.39, indicating a  $P$  value of  $<0.01$ . The scattergram in Fig. 4 shows that two groups of workers fell far outside the regression line. One consisted of the seven workers with symptom scores of 4 or more who were skin test negative or reacted only at a concentration level where reactions of primary irritation could be expected. These workers were all RAST negative. On the other hand, there were twenty-eight workers who had scored symptoms of 4 or less who were highly skin sensitive at much lower concentrations. These workers were, with only two exceptions, RAST positive. It seems likely that the symptoms reported by the first group of workers were of a non-allergic nature since two independent tests were negative while the second group of workers were clearly sensitized individuals in spite of a negative clinical history. It would not be surprising if respiratory symptoms were forgotten by some individuals after 1–3 years. It is also possible that some of these workers had been exposed to enzyme dust levels which caused sensitization but which were not high enough to evoke clinical symptoms. The results clearly demonstrate the difficulty of assessing and comparing symptom severity. One reason may be that individual exposure levels were often changed. Nevertheless, it is evident that the skin sensitivity and RAST values were significantly correlated with the symptoms of this type of allergy.

## Discussion

The recognition of the allergenic potency of airborne *B. subtilis* enzyme dust resulted in a drastic reduction of its use in detergents some years ago. They later reappeared on the market, particularly in Europe. It is to be hoped that prilling and coating

procedures have solved the problem of dust generation in the factories handling these materials.

As an example of the still existing concern for the hazard of sensitization the Swedish Board of Occupational Safety and Health in 1975 has required legal dust control measurements at regular intervals in factories where enzymes are industrially handled and recommended medical follow-up to disclose signs of sensitization. Industrial handling of enzymes also occurs outside the detergent industry with *B. subtilis* and other enzymes. Thus Colten *et al.* (1975) recently reported four cases of occupational allergy to hog-trypsin in a plastic industry: the authors stressed the usefulness of skin testing to disclose sensitization among exposed workers. However, active proteolytic enzymes can be expected *a priori* to give skin irritation when applied or injected in sufficient concentration. The level for such irritation has to be pre-determined when these solutions are to be used as test reagents for specific skin sensitivity. The large weal and flare reactions elicited regularly at the intradermal injection of 100  $\mu\text{g}$  per ml of *B. subtilis* enzyme pools in most non-sensitized controls were undoubtedly due to non-specific irritation. Their appearance was indistinguishable from specific skin test reactions in a large group of sensitized workers. Even at a concentration of 10  $\mu\text{g}$  protein per ml, most control subjects gave weal reactions of a size only slightly smaller than the specific reactions in sensitized workers whose threshold of specific skin sensitivity was shown at a much lower concentration. Discrimination between specific and non-specific intradermal skin test reactions at this 10  $\mu\text{g}$  per ml concentration level might be guided by the accompanying flare reaction. A flare with the weal was much less frequent in the control subjects than in the sensitized workers, but this was not an absolute finding. Because of the difficulty in distinguishing specific and non-specific reactions at 10  $\mu\text{g}$  per ml, only reactions at 1  $\mu\text{g}$  per ml were considered indicative of true allergic sensitivity. This conclusion is supported by the rarity of specific IgE in the serum of workers who reacted only at 10  $\mu\text{g}$  per ml.

Bernstein tested intradermally non-worker populations with one subtilopeptidase A product and one subtilopeptidase B product but considered reactions at higher concentrations (25  $\mu\text{g}$  per ml for subtilopeptidase A and 19  $\mu\text{g}$  for subtilopeptidase B) as indicative of sensitivity (Bernstein, 1972). He did, however, include as positive weal reactions without flare of the sort we have described in controls. He notes that these concentrations of his test substances rarely caused irritant reactions. Similar test concentrations and test enzymes were also used by Dolovich & Little (1972). In non-sensitized control subjects the authors found that their reagents elicited weals of a slightly smaller size than was found in our study. Similarly, they noted the absence of flares as a typical feature of these non-specific reactions. They considered positive only those reactions with a weal size greater than 1 s.d. above the mean weal size found in normals. In order to consider an intradermal reaction specific, we have preferred to insist on a weal *and* flare at a concentration (1  $\mu\text{g}$  per ml) which never caused a reaction in normals.

While non-specific reactions occurred regularly in the controls when the intradermal test concentration exceeded 1  $\mu\text{g}$  protein per ml, non-specific prick test reactions were elicited less regularly by 10 000 times higher test concentration of the enzyme pools (10 mg protein per ml). The irregularity of the false positive prick test reactions in the controls contrasted to the high reproducibility of the specific prick test reactions in the sensitized workers at concentrations 1000 times higher than those

giving positive intradermals. In fact, the specific prick test reactions were almost as reproducible as the intradermal reactions, which may seem surprising, considering the 'blind' way of administering the test reagent with a single puncture lesion. However, the ability of the prick test method to identify specific sensitivity reproducibly is confirmed by this study. Based upon a comparison of skin responses to prick and intradermally administered histamine, Squire calculated that a volume of  $3 \cdot 10^{-6}$  ml is introduced through the prick test lesion (Squire, 1950). Accordingly, a prick test should require a 1,000 times higher concentration to elicit a positive reaction than an intradermal test. The results of this study were in agreement with this estimate and prick test reactions at 1 mg enzyme protein per ml could be considered as specific.

Symptom severity was probably the most difficult parameter to evaluate in the study. Nevertheless, the clinical history as scored by a questionnaire revealing type I respiratory allergy symptoms correlated significantly with the skin sensitivity and RAST result. This fact supports the assumption that *B. subtilis* enzyme allergy is mainly IgE mediated although other types of reaction cannot be excluded by such results.

With both prick and intradermal tests, it was possible to set a level of concentration for testing which would not cause a reaction in controls but would elicit a reaction in sensitized individuals. The validity of this conclusion was supported in a general way by the results of symptom questionnaires and testing for specific circulating IgE-antibodies by RAST. The RAST as performed for this study was somewhat less sensitive than either prick or intracutaneous test, so that many individuals with low levels of sensitivity revealed by skin test were negative by RAST. One laboratory furnished RAST results which correlated closer to skin reactivity than the other. Furthermore the results from this laboratory did not disagree in workers without signs of sensitivity as did some results from the other laboratory in spite of use of the same antigens by both. The difference of precision discloses the difficulty in optimizing this highly sophisticated test system. This points to the advantage of the skin test, although RAST was found to be a precise diagnostic test for low level specific sensitivity to subtilisin-A in a low proportion (1.9%) of a Swedish consumer population (Zetterström & Wide, 1974).

In a few instances at low levels of sensitivity, prick tests revealed evidence of sensitivity less clearly demonstrated by intracutaneous tests. A single prick test with a representative test reagent at appropriate test concentration can disclose a specific IgE sensitivity as precisely but more cheaply and more easily than an intradermal skin test or a RAST test. For occupational health hazards, prick tests should be particularly useful for screening purposes. Intradermal test and RAST will add confirmatory-evidence to establish the diagnosis in selected cases.

In advance of this testing the nature and type of enzyme(s) used by the factory in question should be clearly determined. Alcalase was not always an appropriate test material because it had not been used by all American factories involved in this study. The employed test pools are probably still up to date in the U.S.A. but in the future and in other countries new test reagents may have to be prepared. The results of this study may provide guidance for standardization and test procedures in such situations.

## References

- BELIN, L., FALSEN, E., HOBORN, J. & ANDRE, J. (1970) Enzyme sensitization in consumers of enzyme-containing washing powder. *Lancet*, **ii**, 1153.

- BERNSTEIN, I.L. (1972) Enzyme allergy in populations exposed to long-term, low-level concentrations of household laundry products. *Journal of Allergy and Clinical Immunology*, **49**, 219.
- CARTER, R.O. & McMURRAIN, K.D. (1970) Consumer safety of enzyme detergents. *Journal of the American Medical Association*, **211**, 2017.
- COLTEN, H.R., POLAKOFF, P.L., WENSTEIN, S.F. & STRIEDER, D.J. (1975) Immediate hypersensitivity to hog trypsin resulting from industrial exposure. *New England Journal of Medicine*, **292**, 1050.
- DOLOVICH, J. & LITTLE, D.C. (1972) Correlates of skin test reactions to *Bacillus subtilis* enzyme preparations. *Journal of Allergy and Clinical Immunology*, **49**, 43.
- EDITORIAL (1971) Sensitization to the enzymes in detergents. *Journal of Allergy*, **47**, 53.
- FLINDT, M.L.H. (1969) Pulmonary disease due to inhalation of derivatives of *Bacillus subtilis* containing proteolytic enzymes. *Lancet*, **i**, 1177.
- GREENBERG, M., MILNE, J.F. & WATT, A. (1970) Survey of workers exposed to dusts containing derivatives of *Bacillus subtilis*. *British Medical Journal*, **2**, 629.
- HOW, M.J. & CAMBRIDGE, G.W. (1971) Prick-tests and serological tests in the diagnosis of allergic reactivity to enzymes used in washing products. *British Journal of Industrial Medicine*, **28**, 303.
- LITTLE, D.C. & DOLOVICH, J. (1973) Respiratory disease in industry due to *B. subtilis* enzyme preparations. *Journal of the Canadian Medical Association*, **108**, 1120.
- LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L. & RANDALL, R.J. (1951) Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, **193**, 265.
- NEWHOUSE, M.L., TAGG, B., POCKOCK, S.J. & McEWAN, A.C. (1970) An epidemiological study of workers producing enzyme washing powders. *Lancet*, **i**, 689.
- PEPYS, J., HARGREAVE, F.E., LONGBOTTOM, J.L. & FAUX, J. (1969) Allergic reactions of the lungs to enzymes of *Bacillus subtilis*. *Lancet*, **i**, 1181.
- SHAPIRO, R.S. & EISENBERG, B.C. (1971) Sensitivity to proteolytic enzymes in laundry detergents. *Journal of Allergy*, **47**, 76.
- SLAVIN, R.G. & LEWIS, C.R. (1971) Sensitivity to enzyme additives in laundry detergent workers. *Journal of Allergy and Clinical Immunology*, **48**, 262.
- SQUIRE, J.R. (1950) The relationship between horse dandruff and horse serum antigens in asthma. *Clinical Science*, **9**, 127.
- WEILL, H., WADDELL, L.C. & ZISKIND, M. (1971) A study of workers exposed to detergent enzymes. *Journal of the American Medical Association*, **217**, 425.
- WIDE, L., BENNICH, H. & JOHANSSON, S.G.O. (1967) Diagnosis of allergy by an *in vitro* test for allergen antibodies. *Lancet*, **2**, 1105.
- ZETTERSTRÖM, O. & WIDE, L. (1974) IgE-antibodies and skin test reactions to a detergent-enzyme in Swedish consumers. *Clinical Allergy*, **4**, 273.

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