

Characterization of skin prick testing responses for detecting sensitization to detergent enzymes at extreme dilutions: Inability of the RAST to detect lightly sensitized individuals

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Abstract

We observed that a group of detergent enzyme workers with known exposure to the subtilisin enzyme, Alcalase (Novo Industries, Bagsvaerde, Denmark), exhibited percutaneous sensitivity to Savinase (Novo Industries), a microbial protease, to which there was no previous occupational exposure. This was attributed to either cross-reactivity between these enzymes or to foreign enzyme contaminants contained in the Savinase antigen. The aims of this study were to determine the range of concentrations eliciting percutaneous responses to Alcalase and to another enzyme, Rapidase (an α -amylase) (Gist Brocades, Belgie, Netherlands); to compare the sensitivity of RAST and skin prick testing; and to characterize the relationship between wheal size and antigen concentration. Prick testing was conducted over six $\log_{10}$ antigen dilutions of Alcalase and Rapidase in 30 workers with previous exposure and skin reactivity to enzymes (group 1) and compared to nonexposed control groups, which included 60 atopic subjects (group 2) and 30 nonatopic subjects (group 3). The RAST was performed with Alcalase and Rapidase antigens. The percutaneous threshold concentrations in group 1 subjects varied widely from 10^{-3} to 10^{-3} μ g of protein per milliliter. Of 19 group 1 workers with skin test reactivity to Alcalase, 84% had positive RAST results; 83% of 24 workers who were reactive to Rapidase had positive RAST results. It was concluded that skin prick testing is preferred over in vitro methods for longitudinal monitoring of human sensitization to workplace allergens. In addition, the data predicted that based on a known Alcalase level of 0.07% in Savinase, 26% of Alcalase-sensitized subjects could react to Savinase. An excellent correlation ($r > 0.97$) was found between log concentration of antigen and wheal size parameters, with the log diameter and log area performing equally as well ($r > 0.98$). Analysis of variance revealed that more than 60% of intragroup variation represented human variability in wheal parameters at each concentration tested, whereas at least 95% of intergroup variation was due to regression. The excellent correlations of both wheal diameter and area with

antigen concentrations were attributed to the very small changes observed between test concentrations. (J ALLERGY CLIN IMMUNOL 1994;94:498-507.)

ANOVA Analysis of variance

Key Words: [Enzyme](#), [Alcalase](#), [\$\alpha\$ -amylase](#), [Savinase](#), [prick testing](#), [RAST](#)

Recently, new enzymes from various *Bacillus* strains have become commercially available and are being introduced into detergents. Before its introduction, a group of previously unexposed detergent workers were prick tested with Savinase (Novo Industries, Bagsvaerde, Denmark), one of the newer proteases produced by *Bacillus subtilis lentes*, to establish baseline sensitivity for prospective monitoring. Fifty of 543 (9%) employees exposed to Alcalase (Novo Industries) for many years surprisingly exhibited percutaneous test reactivity to Savinase, although they had no known previous exposure to this newly introduced enzyme.

A similar occurrence was reported by Dor et al.¹ in a separate group of employees who had been exposed to Esperase (Novo Industries), a protease derived from another *Bacillus* species. Serum-specific IgE levels to Esperase were elevated, as expected, but the sera were also reactive to Alcalase and Savinase, although there had been no previous exposure to these enzymes. RAST inhibition studies demonstrated that IgE bound to Savinase was inhibited well by Esperase and Alcalase. These investigators attributed their findings to cross-reactivity between allergenic epitopes of these enzyme antigens rather than to cross-contamination of one enzyme batch by another. These authors thought cross-contamination was unlikely because their antigens were considered to be of extremely high purity (i.e., Esperase contained less than 0.01% Alcalase and less than 1% Savinase; Savinase had 0.1% Alcalase).

In subsequent publications, Arlian et al.² and Sarlo et al.³ were able to show that cross-inhibition between Alcalase and Savinase does not occur. Gallagher et al.⁴ showed that an incompletely inactivated protease could inhibit specific IgE binding to an unrelated short ragweed antigen, suggesting that the cross-reactivity found by Dor et al.¹ could also have been explained by incomplete enzymatic inactivation of inhibiting enzymes. As an alternative explanation for Savinase reactivity in Alcalase-exposed workers, we suspected that the Savinase antigen could contain trace amounts of Alcalase at a sufficient level to elicit positive prick test results in some of Alcalase-exposed workers whom we had observed. To determine whether a very low level of Alcalase contained in the Savinase antigen was sufficient to cause percutaneous reactions in the employees tested for Savinase sensitivity, a dose-response study of prick test sensitivity to Alcalase carried to extreme dilutions was performed. Because enzymes other than proteases are also used in detergents, it seemed appropriate to include Rapidase, an α -amylase, in these dilution studies. Prick test titration data for these enzymes were compared with serum levels of specific IgE antibodies. In addition, complete data pertaining to the wheal and flare response were obtained from prick testing performed over an unusually wide range of six 10-fold antigen dilutions. Previous skin test titration studies with enzymes and other allergens have demonstrated that wheal size increases with concentration. There is not complete agreement as to the best wheal parameter (i.e., wheal size or wheal area) for use in the dose-response curve or the extent of the dilution range in which a linear wheal size response may be found. Data from our study, which examines the relationship between wheal size and antigen concentration, will be presented and used to suggest an explanation for the apparent differences between results of earlier studies.

Methods

Study group

Three subject groups participated. Group 1 included 30 persons with previous work exposure and cutaneous reactivity to detergent enzymes. Group 2 comprised 60 persons who were atopic as defined by a personal history of allergic rhinitis and skin prick test reactivity to two or more common aeroallergens. Group 3 consisted of 30 nonatopic volunteers who were free of symptoms. Both groups 2 and 3 had no known

enzyme exposure at work. All subjects were given explanations of the risks and benefits of the procedures and signed an informed consent statement approved by the I. L. Bernstein, Inc. Institutional Review Board.

Materials

Two commercially available detergent enzymes, Alcalase, a protease produced by *Bacillus licheniformis* (Novo Industries, Bagsvaerde, Denmark) and Rapidase, an α -amylase produced by *Bacillus amyloliquefaciens* (Gist Brocades, Belgie, Netherlands), were used for skin prick test dilution studies. Six 10-fold dilutions were prepared with phosphate-buffered saline on the day of testing from starting concentrations of Alcalase (2.990 mg of protein/ml, Kjeldahl's method) and Rapidase (4.619 mg of protein/ml, Kjeldahl's method); the final diluted solutions in the series contained 2.99×10^{-3} and 4.619×10^{-3} μ g protein/ml, respectively. Starting solutions were sterilized through Millipore filters (Millipore Corp., New Bedford, Mass.). Control skin test reagents included histamine phosphate (1 mg/ml) and phosphate-buffered saline. Skin prick tests were also performed with a panel of 1:20 wt/vol commercial aeroallergens, which included house dust mite (*Dermatophagoides farinae*), *Alternaria* and *Cladosporium* mold allergens, cat dander, short ragweed, and timothy grass and box elder pollens (Hollister-Stier, Spokane, Wash.).

Immunologic studies

Prick tests at each enzyme concentration were performed in duplicate on contralateral sites of each subject's forearm. Approximately 0.05 ml of each test reagent was applied to the skin, followed by puncture with a 27-gauge hypodermic needle.⁵ All test sites were spaced 2.5 cm apart. Separate needles were used for each concentration tested. After 12 minutes, cutaneous wheal and flare responses were circumscribed and transferred with Transpore tape (3M Healthcare, St. Paul, Minn.) to a data form. Both long and orthogonal wheal and flare diameters were measured and recorded. A wheal response that was at least 3 mm in diameter greater than that produced by the negative saline control with a surrounding flare was considered a positive response. The threshold value was defined as the lowest dilution producing a positive prick test response on at least one test site.

Serum-specific IgE to both Alcalase and Rapidase enzymes was measured by a modified RAST assay.³ Alcalase (2.99 mg protein/ml) and Rapidase (4.62 mg protein/ml) enzymes were bound to methylcellulose disks with cyanogen bromide at pH 11 and washed three times in 1 mol/L ethanolamine (pH 8).⁶ As part of the disk preparation procedure, the RAST disks were washed three times in a 0.1 mol/L acetate buffer (pH 4). Such acidic treatment inactivates enzymatic sites on Alcalase and Rapidase and thus eliminates the possibility of enzymatic consumption of IgE in the RAST assay.⁷ The test serum was then incubated with antigen-bound disks for 14 hours and washed with phosphate-buffered saline followed by addition of 35,000 cpm of iodine 125–radiolabeled anti-human IgE. Results of the RAST assays were expressed as counts per minute bound. Values were considered significantly elevated and RAST results were considered positive if greater than 3 standard deviations above the mean binding value of the atopic control subjects (group 2) (Alcalase RAST positive result > 405 cpm; Rapidase RAST positive result > 522 cpm). Serum total IgE was determined by radioimmunoassay in all subjects, and results are expressed as international units (IU) per milliliter.

Data analysis

Wheal diameter was expressed as the sum of the long and orthogonal diameters divided by 2, and the wheal area was approximated as the product of the long and orthogonal diameters. The statistical methods used for computing the regression of wheal size with concentration, when individual rather than group mean values of wheal size at each test concentration were analyzed, are described by Sokal and Rolf.⁸

Results

Skin test reactivity to Alcalase and Rapidase was observed predominantly in group 1 workers with previously documented prick test reactivity to these enzymes. In group 1 the mean of the wheal diameter elicited by skin prick testing with 1 mg/ml histamine phosphate was 5.7 ± 1.4 mm. The prick method was reproducible as demonstrated by a mean percent variability of 16.3 ± 12.9 in the sum of the long and orthogonal wheal diameters between duplicate Alcalase tests in group 1 subjects with skin reactivity to Alcalase. In this group,

three workers with prior skin reactivity to these enzymes had negative responses at the time of testing; 27 of 30 (90%) had positive prick test responses to at least one enzyme, and 16 of 30 (53%) reacted to both enzymes. Nineteen of 30 (63%) had positive prick test responses to Alcalase, and 24 of 30 (80%) had positive responses to Rapidase. Three (10%) reacted exclusively to Alcalase, and eight (27%) reacted exclusively to Rapidase.

The ranges of threshold concentrations that elicited percutaneous responses to either of these enzymes in group 1 subjects are shown in ■Table I; intersubject threshold responses varied widely (from 10^3 to 10^{-3} µg of protein/ml). Five workers showed reactivity at concentrations of Alcalase of 10^{-3} µg protein/ml. Among the 27 workers with percutaneous reactivity to at least one enzyme, there were 10 (37%) who could be classified as atopic according to the definition used in this study. Data for the 90 subjects in groups 2 and 3 are also shown in ■Table I. A subject in group 2 was clearly sensitized with threshold responses of 10^{-2} and 10^{-3} µg to Alcalase and Rapidase, respectively. Subject 2 in group 2 and subject 2 in group 3 had positive test responses to Alcalase at the highest concentrations tested, and subject 1 in group 3 had a positive test response to Rapidase at the highest concentration.

TABLE I. Skin prick threshold responses* to Alcalase and Rapidase

	Subject no.	Alcalase*	Rapidase*
Group 1	1	-3	0
	2	0	3
	3	1	-1
	4	-3	-1
	5	NR	2
	6	-1	0
	7	NR	2
	8	-3	0
	9	-2	0
	10	0	0
	11	-3	0
	12	-1	1
	13	-1	-1
	14	-2	-1
	15	-3	-2
	16	-2	2
	17	NR	-1
	18	NR	0
	19	2	3
	20	NR	3
	21	NR	1
	22	2	NR

	23	NR	NR
	24	3	NR
	25	NR	1
	26	NR	0
	27	1	0
	28	1	NR
	29	NR	NR
	30	NR	NR
Group 2 (n= 60)	1	-2	-3
	2	3	NR
	3-60	NR	NR
Group 3 (n= 30)	1	NR	3
	2	3	NR
	3-30	NR	NR

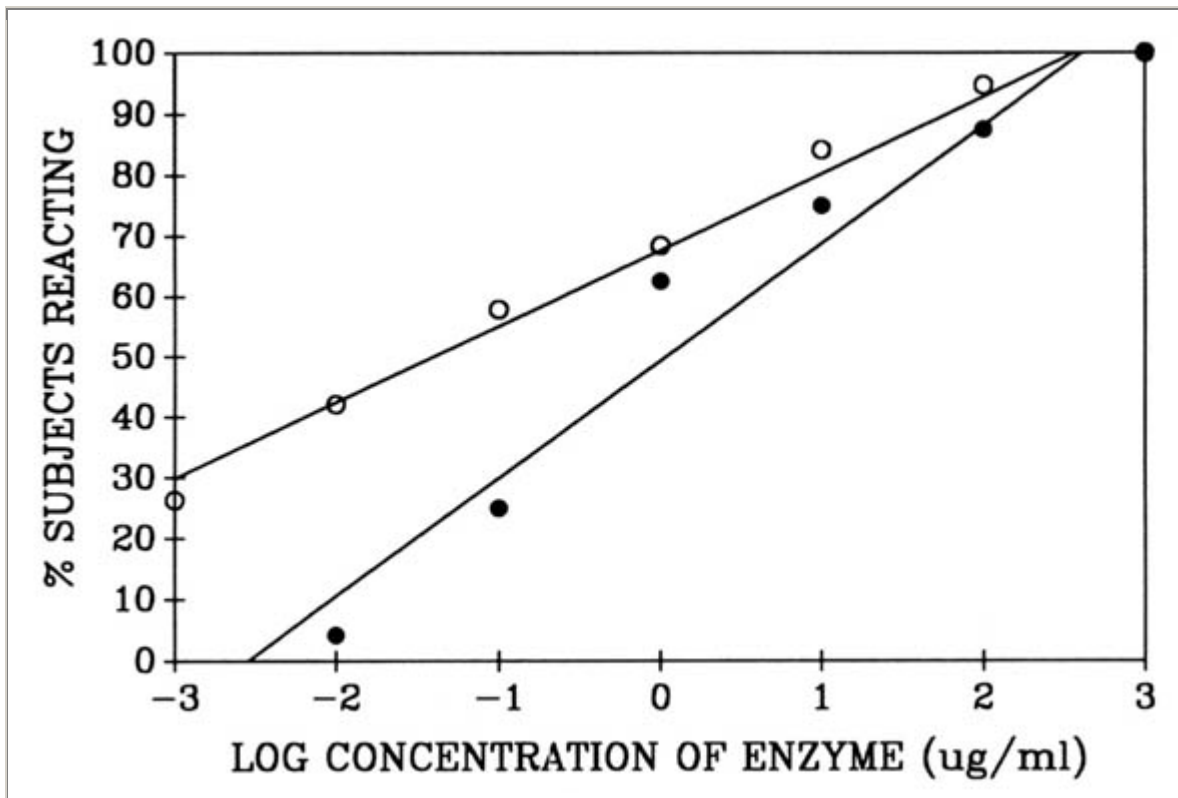
Group 1, enzyme-exposed and previously sensitized; group 2, atopic with no enzyme exposure; group 3, nonatopic with no enzyme exposure.

NR, No reaction.

*Concentration expressed as coefficient (x) of $2.99 \times 10^x \mu\text{g}$ protein/ml for Alcalase and of $4.62 \times 10^x \mu\text{g}$ protein/ml for Rapidase

In ■Fig. 1, the relative percentage of workers with threshold levels at or below a certain dilution decreased in a linear fashion in proportion with the decrease in log antigen concentration ($r=0.992$ for Alcalase; $r = 0.976$ for Rapidase). Visual inspection of these data, and the very high correlation coefficients, show that the fit to a least squares straight line is excellent for both enzymes and permits estimation of the proportion of employees expected to have positive skin test results at each log concentration. These data also show that significant proportions of the employees had positive skin test responses to solutions that had been diluted 5 to 6 orders of magnitude below the usual testing concentration.

FIG. 1. Percentage of subjects in group 1 exhibiting prick test reactivity at or below various $\log_{10}$ dilutions of $2.990 \mu\text{g}$ of protein/ml Alcalase (*open circles*) versus the concentration of Alcalase used for prick testing ($r = 0.992$; $p < 0.001$); percent of positive prick test results at a given concentration of Alcalase = $67.7 + 12.6 \log C$. Percentage of subjects with prick test reactivity at or below various $\log_{10}$ dilutions of $4.619 \mu\text{g}$ protein/ml of Rapidase (*filled circles*) ($r = 0.976$, $p < 0.001$). Percent of positive prick test results at a given concentration = $49.3 + 19.4 \log C$.



The mean RAST values for the exposed group 1 workers for Alcalase (809 ± 732 cpm) and Rapidase (2463 ± 2657 cpm) were significantly greater than those found for the group 2 unexposed and atopic subjects (207 ± 108 cpm, $t = 6.16$, $p < 0.001$ for Alcalase and 252 ± 90 cpm, $t = 6.89$, $p < 0.001$ for Rapidase). Group 1 also differed significantly from the group 3 nonatopic subjects (259 ± 62 cpm, $t = 4.10$, $p < 0.001$ for Alcalase and 311 ± 87 cpm, $t = 4.81$, $p < 0.001$ for Rapidase). No subject in either group 2 or group 3 exhibited positive RAST results. As expected, mean serum total IgE was lowest in group 3 (30 ± 35 IU/ml) as compared with the atopic control group 2 (181 ± 202 IU/ml) and the enzyme-exposed group 1 (388 ± 1285 IU/ml).

As shown in ■Fig. 2, workers with percutaneous reactivity to Alcalase at or below threshold concentrations of 2.99×10^{-1} μg protein/ml had positive RAST results. As the percutaneous threshold concentration increases, negative RAST results are apparent. Of the eight workers with positive prick test results and threshold concentrations higher than 10^{-1} , three (38%) had negative RAST results. A similar observation was found to be true for Rapidase. As shown in ■Fig. 3, five (56%) of nine workers with Rapidase skin test reactivity to a threshold concentration of 4.6×10^1 $\mu\text{g}/\text{ml}$ or greater did not exhibit elevated RAST binding. A single exception was a worker whose prick test threshold concentration to Rapidase was 10^2 μg protein/ml and who had the highest elevation in RAST binding among all subjects (10,851 cpm). For those individuals who had prick test threshold values above the critical values of 10^{-1} for Alcalase and 10^0 for Rapidase, eight of 17 (47%) would have been considered nonsensitized according to RAST results alone.

FIG. 2. The prick test threshold expressed as the coefficient x of 2.99×10^x μg protein/ml of Alcalase is plotted versus RAST binding as counts per minute (cpm) in those group 1 subjects with cutaneous sensitivity to the enzyme. The RAST result was considered elevated at a binding value greater than 3 standard deviations above the mean binding value of 405 cpm (asterisk) of the control group.

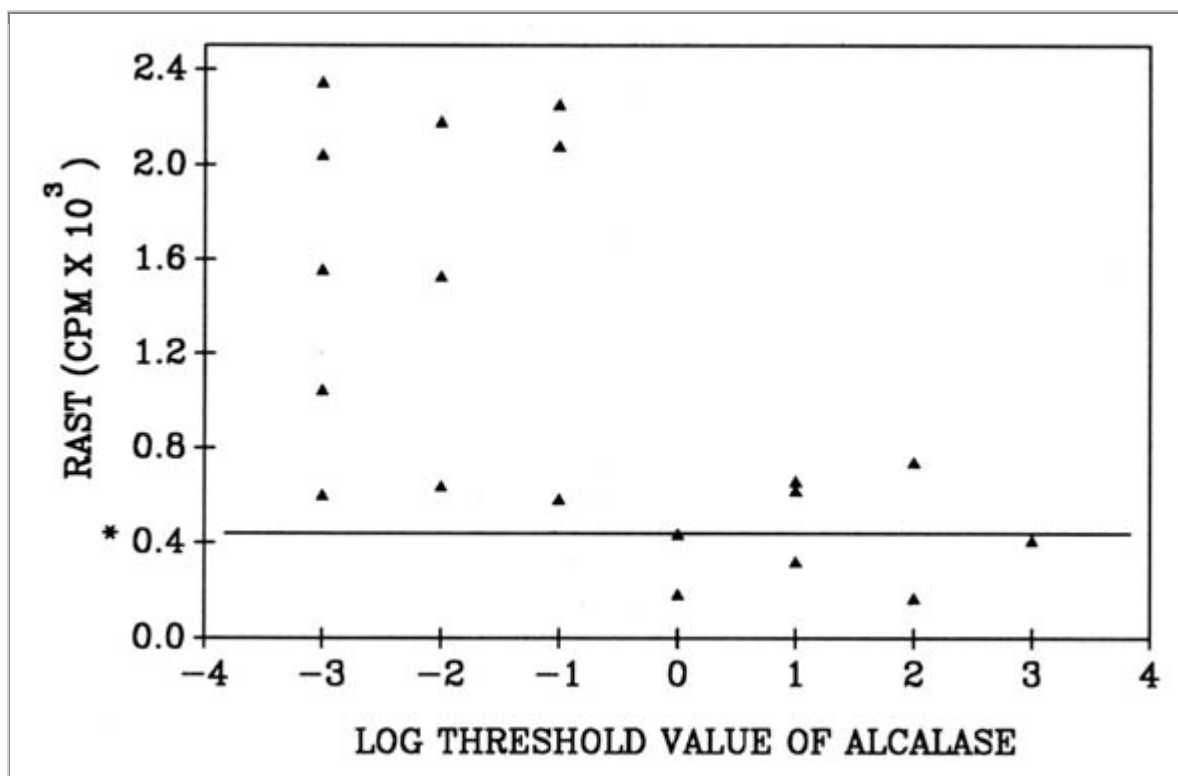
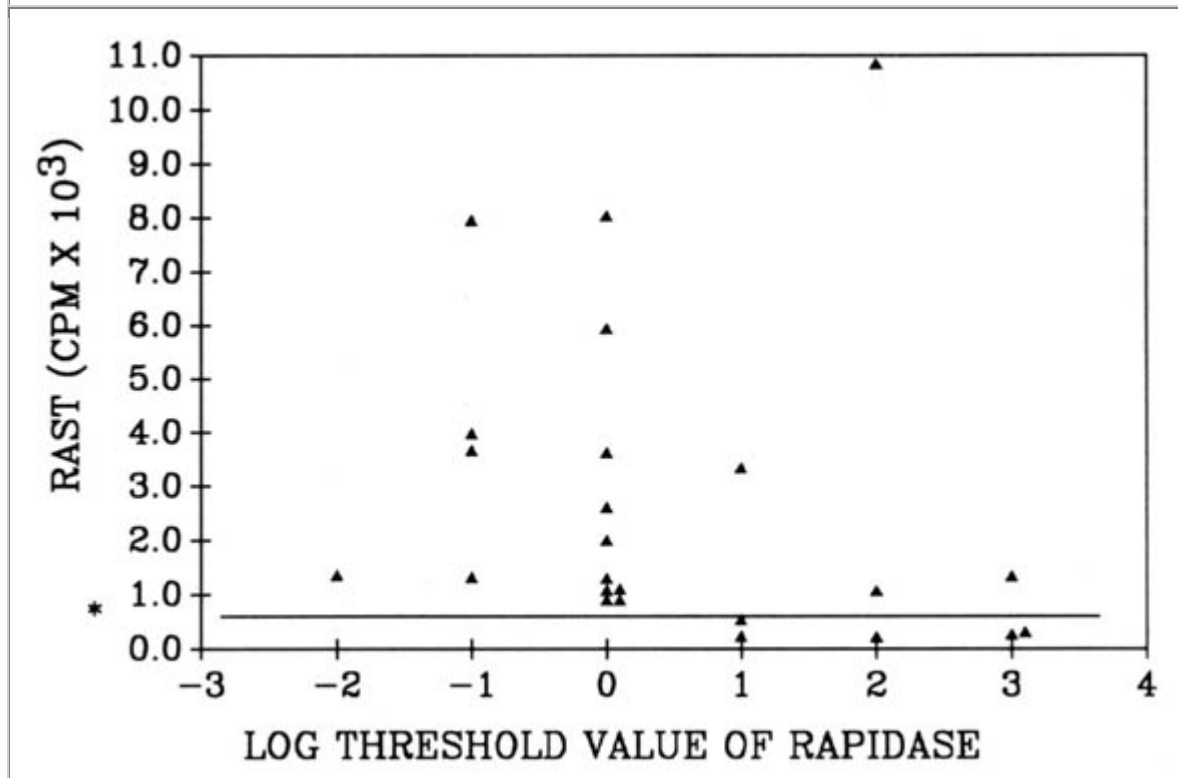


FIG. 3. The prick test threshold expressed as the coefficient x of $4.62 \times 10^x \mu\text{g}$ protein/ml of Rapidase is plotted versus RAST binding as counts per minute (*cpm*) in those group 1 subjects with cutaneous sensitivity to the enzyme. The RAST result was considered elevated at a binding value greater than 3 standard deviations above the mean binding value of 522 cpm (*asterisk*) of the control group.



In this study correlation coefficients between RAST binding and both wheal area and wheal diameter were calculated at log testing concentrations of 10^3 , 10^2 , and 10^1 . The highest correlation coefficients for both enzymes were found at log concentrations of 10^2 : for Alcalase wheal area, $r = 0.508$ ($p < 0.001$); for Rapidase wheal area, $r = 0.518$ ($p < 0.001$); for Alcalase wheal diameter, $r = 0.517$ ($p < 0.001$); and for

Rapidase wheal diameter, $r = 0.496$ ($p < 0.001$). These values are very similar to the correlation coefficients reported earlier for Alcalase by Wilson ($r = 0.5$) and How ($r = 0.6$).⁹

Differences have existed among investigators as to whether the best fit to a straight line is obtained by plotting mean wheal size or log mean wheal size versus log concentration of the test solution. Our data for Alcalase, treated in each of these ways, are shown in ■Figs. 4 and ■5. The fit to a straight line, as determined by the correlation coefficients ($r \geq 0.97$), would be considered excellent by any of the methods of graphing. The coefficient of determination (r^2) is a better measure of the fit to the regression line. With this measure, the best fits for the Alcalase data were obtained with the log-transformed mean wheal diameter and area. In addition, it is obvious from ■Fig. 4 that the data in each graph would better fit a curved line rather than the least squares regression lines shown, despite the very high correlation coefficients. Graphs of the log/log relationships shown in ■Fig. 5 form essentially straight lines, confirming that the log/log relationship provides a better fit to a straight line. These data do not show a difference or superiority between the log diameter or log area as a measure of the wheal size.

FIG. 4. Plot of mean wheal diameter (in millimeters) versus log concentration of Alcalase (*filled circles*) ($r = 0.989$; $r^2 = 0.979$; $p < 0.001$). The x axis represents the coefficient x of 2.99×10^x μg protein/ml of Alcalase. Plot of mean area of wheal versus log concentration of Alcalase (*open circles*) ($r = 0.98$; $r^2 = 0.952$; $p < 0.001$).

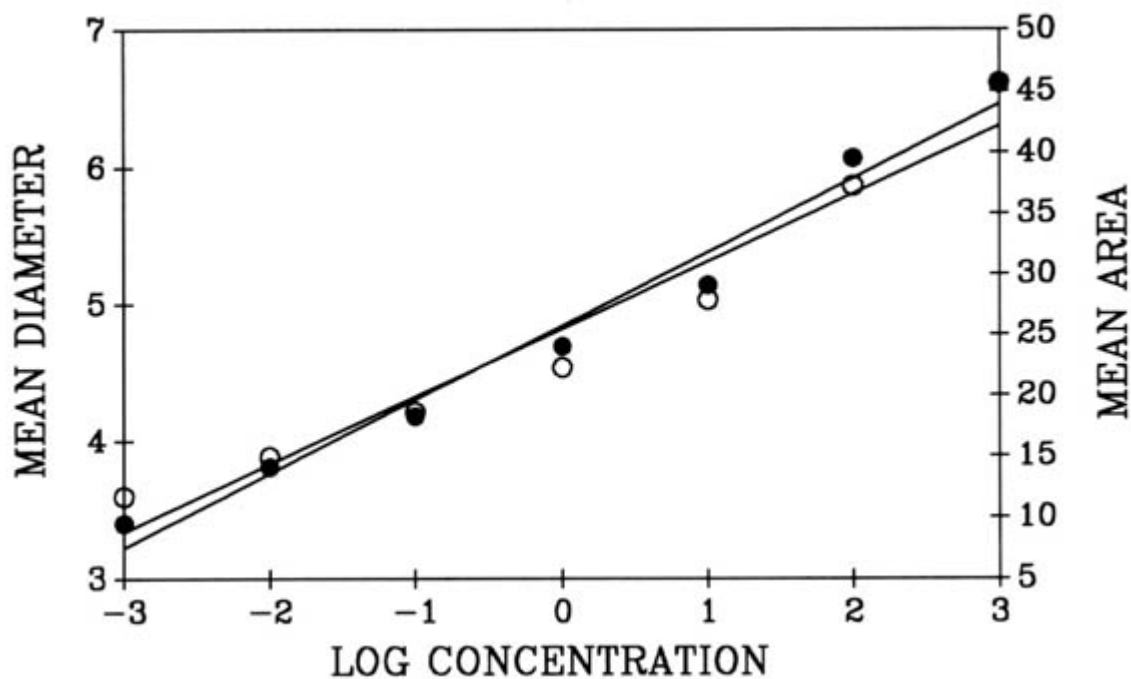
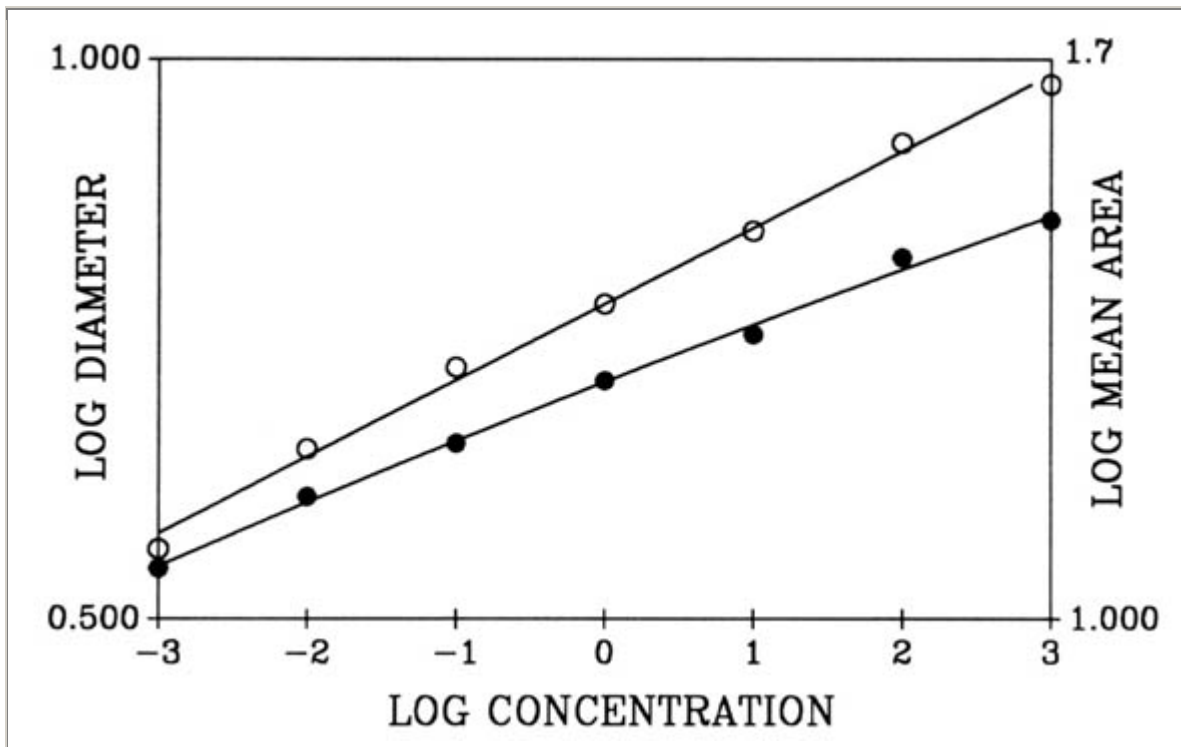


FIG. 5. Plot of log mean diameter (in millimeters) versus log concentration of Alcalase (*filled circles*) ($r = 0.99$; $r^2 = 0.995$; $p < 0.001$). The x axis represents the coefficients x of 2.99×10^x μg protein/ml of Alcalase. Plot of log mean area of wheal versus log concentration of Alcalase (*open circles*) ($r = 0.99$; $r^2 = 0.997$; $p < 0.001$).



A plot of each individual's wheal diameter versus log concentration of Alcalase is shown in ■Fig. 6. Although the data will produce the regression line shown, it is clear that there are substantial individual variations within each enzyme concentration. Regression statistics were used in combination with analysis of variance (ANOVA) to prepare a full ANOVA table evaluating the variability of the Alcalase data (■Table II). ■Table II shows that 65% of the total variation in wheal diameter occurs within the groups and represents human variability at each concentration tested. Of the 34.6% variation that occurs between groups, 98% of it is due to regression. However, as seen from ANOVA data in ■Table II, the log diameter provides a possibly better fit than the diameter because 99% of the variation between groups is explained by regression. As also shown in ■Table II, a similar result was obtained from an identical analysis of the Alcalase wheal areas. The ANOVA of Rapidase wheal data (not shown) also provided similar results.

FIG. 6. Plot of wheal diameter (in millimeters) of individual workers at each given log concentration of Alcalase. The x axis represents the coefficient (x) of 2.99×10^x μg protein/ml of Alcalase. The least squares regression line is drawn.

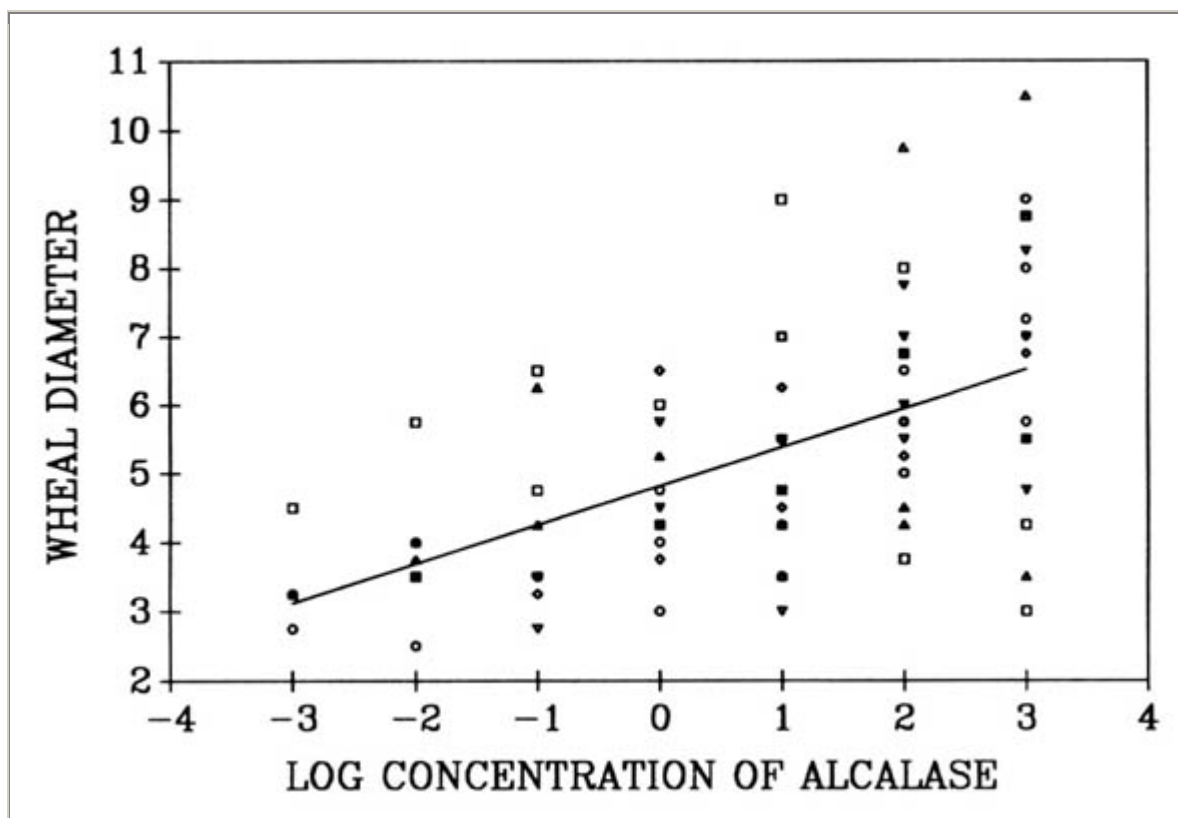


TABLE II. ANOVA including regression for Alcalase

Source of Variation	df	SS	MS	F	Explained
<i>ANOVA diameter of wheal vs log concentration</i>					
SS between groups	6	94.0338	15.6731	6.97	34.6%
SS due to regression	1	91.8851	91.8851	213.33	97.7%
SS deviation from regression	5	2.1536	0.4307	0.19	2.3%
SS within groups	79	177.6851	2.2492		65.4%
SS total	85	271.7238			
<i>ANOVA log diameter of wheal vs log concentration</i>					
SS between groups	6	0.6523	0.1087	7.72	37.0%
SS due to regression	1	0.6433	0.6433	354.71	98.6%
SS deviation from regression	5	0.0091	0.0018	0.13	1.4%
SS within groups	79	1.1128	0.0141		63.0%
SS total	85	1.7652			
<i>ANOVA area of wheal vs log concentration</i>					
SS between groups	6	10863.68	1810.64	5.82	30.7%
SS due to regression	1	10348.62	10348.62	100.43	95.3%
SS deviation from regression	5	515.24	103.05	0.33	4.7%
SS within groups	79	24565.46	310.96		69.3%

SS total	85	35429.31			
<i>ANOVA log area of wheal vs log concentration</i>					
SS between groups	6	2.5127	0.4188	7.46	36.2%
SS due to regression	1	2.4817	2.4817	401.50	98.8%
SS deviation from regression	5	0.0309	0.0062	0.11	1.2%
SS within groups	79	4.4322	0.0561		63.8%
SS total	85	6.9449			

df, Degrees of freedom; SS, sum of squares; MS, mean square; *F*, F ratio.

Discussion

The validity of a skin prick test result depends on several factors. First, the concentration of the antigen in the test solution must be high enough to cause a detectable wheal and flare response in the majority of sensitized subjects but must not be so high as to cause irritant or nonspecific “false-positive” responses among nonsensitized subjects. Second, the antigen must not be contaminated with other antigens to which an individual may also be sensitized, thereby rendering a “positive” test result uninterpretable. Finally, the antigen must be specific and not cross-react with immunologically similar allergens to which the patient has been previously sensitized.

Most antigens used in allergy testing are not pure. Often, they are mixtures of many components extracted from naturally occurring materials, which could elicit nonspecific or irritant skin reactions. Such effects were reported in 1977 by Belin and Norman¹⁰ who used mixtures of detergent enzymes from a variety of manufacturers at various enzyme concentrations for prick testing of 100 nonsensitized subjects. Nonspecific irritant reactions to subtilisin A protease enzymes occurred at 10,000, 1000, and 100 µg protein/ml in 54.5%, 13%, and 3% of subjects, respectively. On the basis of these results, the sponsor of this study selected 500 µg protein/ml as the optimal skin test concentration to be used in screening employees for enzyme sensitization. In our study skin reactivity was identified in three subjects believed to be nonexposed. Two of the latter subjects showed skin reactivity only at the highest test concentration, which suggested that these were nonallergic irritant responses. The third subject was clearly sensitized to Alcalase and Rapidase.

Numerous studies have examined variation in individual skin prick test responses at various antigen dilutions. This study differs from earlier reports in that a much wider range of antigen concentrations has been studied.¹¹⁻¹⁷ Even at the lowest Alcalase concentration of 2.99×10^{-3} µg protein/ml, 26.3% of workers sensitized to Alcalase had positive skin test results. It is possible that the five workers with skin test reactivity at 10^{-3} µg protein/ml concentrations of Alcalase may have had even lower thresholds because this was the lowest concentration tested. Assuming that the relationship between percent positive prick test results and log concentration continues to be linear, it can be predicted that even a dilution of 2.99×10^{-5} µg/ml protein, 4.7% of Alcalase-sensitive subjects would still have a positive test result. Thus we have confirmed earlier observations that human prick test reactivity varies over many orders of magnitude, and the range of that response has been extended to include at least six 10-fold dilutions.

In their monitoring of employees who were thought never to have been exposed to a new enzyme, Savinase, the company sponsoring this study used a Savinase reagent, which was thought to be pure for percutaneous testing. Subsequent analyses revealed that the Savinase test antigen contained trace amounts (0.07%) of Alcalase. With an Alcalase impurity level of 0.07%, a normal prick test solution of the Savinase antigen prepared at a concentration of 500 µg protein/ml would contain 0.35 µg protein/ml Alcalase. With the use of the data and the regression equation in Fig. 1, it is estimated that 62% of Alcalase-sensitized individuals who were tested with Savinase antigen could have reacted to the Alcalase contained in the Savinase reagent at this concentration. This offers a plausible explanation for the high frequency of unexpected skin test responses to Savinase among employees who had been previously exposed to and possibly sensitized to Alcalase but not to Savinase.

In this study 84% of group 1 workers with percutaneous reactivity to Alcalase had positive RAST results; 83% of workers with cutaneous reactivity to Rapidase had positive RAST results. These values support the results in previous studies, which led a special task force under the sponsorship of the National Institute of Allergic and Infectious Diseases to conclude that the skin prick test is a more sensitive method than the RAST for detection of specific IgE responses.¹⁸

In earlier studies, which primarily used the intracutaneous method, the relationship of wheal size to concentration of the testing solution was evaluated extensively.¹⁹ Miller et al.²⁰ reported a linear response for intracutaneous wheal diameter versus log concentration of antigen over a small range of concentrations. In 1948, Becker and Rappaport²¹ reported a log/log relationship between the greatest wheal diameter and concentration of antigen over four 10-fold dilutions. More recently, Voorhorst and Van Krieken²² reported a series of intracutaneous dilution studies in which a linear association was found between the flare size and the log concentration of antigen over four 10-fold dilutions. The response at either end of the range appeared to depart from a straight line.

With percutaneous skin testing, investigators found a predominantly log/log relationship between wheal size and antigen concentration,^{12, 14-16} a finding that was in agreement with results of most earlier intracutaneous studies.^{21, 22} As in the intracutaneous studies, a very small increase in wheal size was found for each log₁₀ increase in concentration. Thus the earlier studies have shown that the skin test threshold response varies widely and that a linear relationship exists between wheal (or flare) size and concentration. A few investigators reported that the wheal/concentration association best fits a straight line. Most found that either the wheal/log concentration or the log wheal/log concentration relationship provides the best fit. Unlike previous studies, which showed that the straight portion of the line was limited to a few log concentrations, our study greatly extended the concentration range to six 10-fold dilutions. The log versus log plot was straight over the entire range of concentrations and showed a uniform increase in the log mean wheal diameter of 0.049 mm for Alcalase and 0.047 mm for Rapidase for each 10-fold increase in concentration of the testing solution.

With this extended study, we found very high correlation coefficients for associations between both wheal size (diameter and area) versus log concentration and log wheal size versus log concentration. The data from this study suggest that there is no significant difference in the correlation coefficients (*r*) obtained between these two methods of plotting the data. The small regression coefficient (the slope) may explain why such good correlation coefficients can be obtained by different measurements of wheal size. The flatter the slope, the less difference it makes as to how the wheals are measured, and therefore any measure of wheal size when plotted versus log concentration will yield a high correlation coefficient.

In nearly all previous studies, correlation statistics were used to examine relationships between the mean wheal size and log concentrations of antigens. However, this may not be the correct statistical approach. Justification for the use of correlation statistics requires that both the concentration and the wheal size be considered as continuous variables. However, in dilution studies the concentration is set by the investigator and is therefore not a true variable. As done here, the correct statistical analysis would require use of regression statistics in combination with ANOVA to quantitate the sources of variation within the data. On the basis of ANOVA and despite the wheal size variability among subjects at each antigen concentration, the change of log wheal diameter or area as a function of log₁₀ antigen concentration is almost entirely explained as regression along a straight line.

In summary, the range of percutaneous threshold responses to allergens is much greater than previously shown and varies by at least 6 log₁₀ orders of magnitude. Dilution studies over six 10-fold dilutions have confirmed that the wheal size produced by the prick test regresses with the log concentration of the test allergen and that any measure of wheal size provides a high correlation coefficient because of the small change in wheal size from higher to lower antigen dilutions. Our data suggest that the best fit to a straight line occurs when the log/log relationship is used. In our study the RAST detected "highly" sensitized subjects but failed to detect about half of the "lightly" sensitized subjects. We conclude from these data that skin prick testing is the preferred method for longitudinal monitoring of occupational groups who are at risk for sensitization to workplace allergens.

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