

Dose response relationship of allergen, histamine, and histamine releasers in skin prick test and precision of the skin prick test method

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The aim of the present investigation was to study the dose response relationship of allergen, histamine and histamine releasers in skin prick test (SPT) and the precision of the SPT method. In one experiment timothy allergen, histamine HCl, rabbit anti-human-IgE and compound 48/80 were studied in seven patients. In a second experiment timothy allergen and histamine and dog allergen and histamine were investigated in two groups of 10 patients. Histamine HCl 1 and 10 mg/ml induced weals about 15 and 25 mm² (4.5 and 5.5 mm in diameter), respectively. The precision as expressed by the coefficient of variation was about 25 % for histamine and 40 % for allergen for weal areas > 10 mm². Calculations of the regression lines to test the dose response relationships were based on the method of least squares. The best fit was to a log/log model. The slopes of allergen, histamine and histamine releasers were essentially parallel within patients. The median slope of allergen was estimated to about 0.4 based on weal areas and 0.2 based on mean weal diameters. Furthermore, no significant differences were found between the lower and upper parts of the dose response curves of allergen and histamine, although there was a tendency towards steeper slopes at lower concentrations. These results show that histamine concentrations ≥ 1 mg/ml should be used as positive control in SPT and that histamine releasers do not offer advantages over histamine as reference substances in SPT. A common slope for the dose response relationship of allergen and histamine can be used for the estimation of skin sensitivity.

Key words: allergen; anti-human-IgE; compound 48/80; dog; dose response relationship; histamine; precision; skin prick test; timothy.

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CLINICAL ASPECTS

A 10-fold increase in the concentration of allergen, histamine and histamine releasers increases the weal diameter with a factor about 1.5 (e.g. 3; 4.5; 6.8 mm in diameter for three 10-fold dose increases). This means that minor differences in potency between allergen extracts or in sensitivity between patients are difficult to identify with routine skin prick tests. As the precision of the skin prick test method is poor, especially at low responses, the use of 3 mm in weal diameter is recommended as a lower limit for positivity. Since the dose response relationships of allergen and histamine were essentially parallel over a wide range of concentrations, it is possible to use histamine 1 or 10 mg/ml as reference substance in SPT.

In biological standardization (BS) the potency of allergen extracts is estimated in samples of patients clinically sensitive to the allergen in question (1, 11). In contrast to other forms of potency determinations of allergen preparations

this procedure allows comparisons of relative potencies between allergen preparations prepared from different source materials. The Committee on Allergen Standardization within the Scandinavian Society of Allergology proposed

the use of skin prick testing (SPT) as a simple and safe means for BS (1). They also proposed the use of histamine as a reference substance to minimize the influence of differences in SPT technique between clinics and regions. The use of a unit for allergen potency, 1 HEP (Histamine Equivalent in Prick testing) (1) equal to 1000 biological units per ml (BU/ml) (11) was suggested. When practical studies with BS were initiated, it was soon found that the test results were less reliable for low concentrations of allergen extracts in relation to patient sensitivity than for high concentrations (2, 3).

To evaluate the possibility of using higher concentrations of histamine and allergen, we have studied the dose response relationship of allergens and histamine in SPT and the precision of the SPT method. In a preliminary experiment histamine releasers were also included.

MATERIAL AND METHODS

Patients

Three groups of patients were studied in two experiments:

Experiment I. In the first series of experiments the dose responses in SPT to timothy pollen allergen, histamine, compound 48/80 and rabbit anti-human-IgE were studied. Seven (six for compound 48/80) otherwise healthy volunteers with grass pollinosis were included. The mean age was 28 (range 20–38).

Experiment II. In the second series, 10 otherwise healthy volunteers with grass pollinosis (6 men and 4 women) were included. The mean age was 28 (range 20–38). None of these patients was included in experiment I. Furthermore, 10 otherwise healthy volunteers with dog allergy were included. The mean age of patients was 27 (range 18–37).

Skin test material

In the first series of experiments, histamine HCl 10, 3, 1, 0.3 and 0.1 mg/ml was delivered

by the hospital pharmacy. 10 mg/ml of histamine dihydrochloride corresponds to 54.3 mmol/l or 6.3 mg/ml of histamine base. Timothy pollen allergen preparation, 100,000 BU/ml (Pharmalgen®, batch No. DI 9359) was obtained from the manufacturer (Pharmacia AB, Uppsala, Sweden). Rabbit anti-human-IgE was obtained from Dako, Copenhagen, Denmark and compound 48/80 from Professor Kjell Strandberg (National Board of Health and Welfare, Uppsala, Sweden), in the concentration 10 mg/ml. All these materials were reconstituted and diluted in Albumin diluent® (0.03 % albumin human, 0.9 % saline, 0.4 % phenol) obtained from the manufacturer (Pharmacia AB, Sweden). Reconstitution and dilution was performed on the day of the test. Disposable needles, 20 G, were used for the SPT.

In the second series of experiments, timothy allergen preparation (Pharmalgen, batch No. GH 23,601) 100,000 BU/ml and dog allergen preparation (Pharmalgen®, batch No. HI 27,265) 100,000 BU/ml were used. Both materials were reconstituted in 0.9 ml of Albumin diluent to obtain the highest concentration 500,000 BU/ml. A second vial of each preparation was reconstituted with 4.5 ml of diluent to obtain the concentration 100,000 BU/ml, which was then diluted in five-fold steps to obtain a total of seven dilutions (500,000), 100,000, 20,000, 4,000, 800, 160, and 32 BU/ml). Histamine 100 mg/ml (54.3 mmol/l) was obtained from the manufacturer (Pharmacia AB, Sweden, batch No. 820428) and used in both experiments. The histamine was diluted in five-fold steps to obtain six concentrations (100, 20, 4, 0.8, 0.16, and 0.032 mg/ml). All dilutions were made with Albumin diluent (Pharmacia AB, Sweden, batch No. HK 74839) at the day of the test. Prick test needles of the Østerballe type (9) were obtained from the manufacturer (Pharmacia AB, Sweden).

One hundred thousand BU/ml of the timothy allergen preparation corresponds to about 2,000 Allergen Units (AU)/ml according to the US standard and to about 2,000 International Units (IU)/ml compared to the WHO International Standard (IS), as determined by RAST-inhibition. The corresponding data for the dog

allergen preparation were about 200,000 AU/ml and 100,000 IU/ml (suggested IS).

Skin prick test method

In the first series of experiments (1980) duplicate SPTs with each concentration were performed according to Pepys (13). The tip of a 20 G disposable needle was inserted at an angle of 20° to the surface through a drop of test solution. The tip was gently lifted to allow a minute amount of allergen to penetrate into the superficial layer of the skin. One test with each concentration was allocated to the left side and the other to a mirror position on the right side of the back. Tests were performed with all test solutions simultaneously.

In the second series of experiments (1982), the patients were tested with all concentrations in quadruplicate, i.e. 28 tests per patient. When this experiment was performed, there was evidence that the conjunctival sensitivity did not change significantly within 3 weeks (10), despite a significant variation in skin sensitivity with season had been found by Haahtela (6). Therefore, tests were performed twice with a 5-day interval. Later, Taudorf et al. (15) have found no change in the response to three different concentrations of histamine within a 3-week period. On the first occasion tests were performed with allergen and on the second occasion with histamine. All concentrations were tested in quadruplicate. One test with each concentration was allocated to each of four quadrants on the upper part of the back of the patient. SPTs were performed according to the method described by Østerballe & Weeke (12). A needle with a 1 mm tip was pressed against the skin surface at 90°, through a drop of test solution. The same pressure was applied every time.

The test sites were at least 4 cm apart in all experiments.

After 15 min the contours of the weals were encircled by means of a fine filter tip pen. Transparent tape was placed on the site of the drawings, and a slight pressure was applied to the tape. The tape was then transferred to a registration sheet.

In the first series of experiments the longest and the midpoint orthogonal diameters were measured and the geometric mean of the four diameters obtained for the two weals of each concentration was used in the calculations.

In the second and third series of experiments the inner side of the drawings was followed with a digitizer, connected to a VAX computer. The areas were registered and used for further calculations.

Statistical analysis

In the first experiment, the model $\log D = a + b \log (\text{conc})$ was used, where D denotes the geometric mean of the four diameters obtained in duplicate tests, a the intercept with the Y-axis and b the slope of the dose response relationship. The individual slopes were calculated. The slopes were found essentially parallel. The geometric mean of all diameters obtained with the same concentration and substance in all patients was used to illustrate the dose response relationship. Weals with a mean diameter < 2 mm were regarded as negative and excluded from the calculations. The precision was calculated as the coefficient of variation of the two weals (arithmetic mean of diameters of each weal) with the same concentration. All tests with the same concentration were allocated to the same class (response level) on the basis of the mean weal response.

In the second series of experiments, weal areas < 3 mm² (about 2 mm in diameter) were regarded as negative (Fig. 1). The precision was low for response levels < 10 mm² (about 3.5 mm in diameter), mainly because of negative (< 3 mm²) responses. Therefore, weals with an area < 7 mm² (about 3 mm diameter) were regarded as negative and excluded from the estimation of the dose response relationship. Finally, when discussing the response to different concentrations, weal areas < 7 mm² (about 3 mm in diameter) were regarded as negative. However, in Fig. 1 a - d, 3 (negative in all respects) as well as 7 mm² are indicated.

The dose response curves for timothy, dog and histamine were calculated using the models $\log (A) = a + b \log (\text{conc})$, $A = a + b \log$

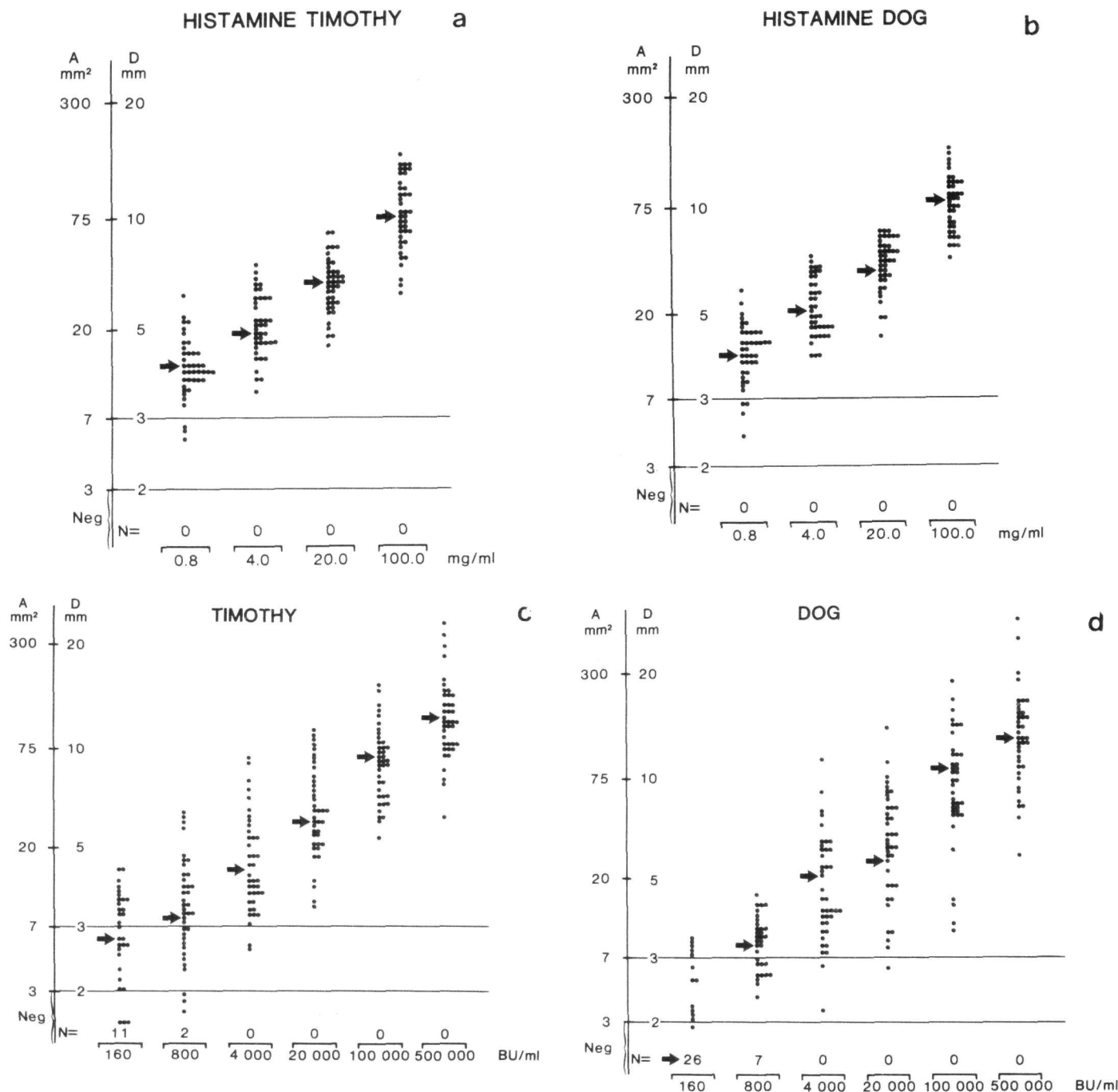


Fig. 1. The individual (filled circles) and the median (horisontal filled arrows) weal areas for histamine (a and b), dog (c) and timothy allergen (d) in the second series of experiments. On the Y-axis are the weal area (log scale) and the corresponding estimated diameters of circular weals. On the x-axis are the concentrations of histamine HCl in mg/ml and allergen in BU/ml. The horizontal lines indicate the true negative tests (< 3 mm² or 2 mm in diameter) and the limit for positivity in some calculations (< 7 mm² or 3 mm in diameter). N denotes the number of negative tests.

(conc) and $\sqrt{A} = a + b \log (\text{conc})$, where A means the geometric mean of the four areas obtained from quadruplicate tests, a the intercept with the Y-axis and b the slope.

The pooled coefficient of variation of the four weal areas obtained with the same concentration was used as a measure of the precision of the SPT method. Calculations were performed with the negative responses (< 3 mm²

or 2 mm in diameter) included and excluded (Fig. 3). Responses were classified into areas 0–10, 10–20, 20–50, 50–75, and > 75 mm² corresponding to about 0–3.5, 3.5–5, 5–8, 8–10, and > 10 mm in diameter based on the median of parallel tests with one concentration in one patient. All test results obtained with one concentration in the same patient were allocated to the same class. To investigate if the area of

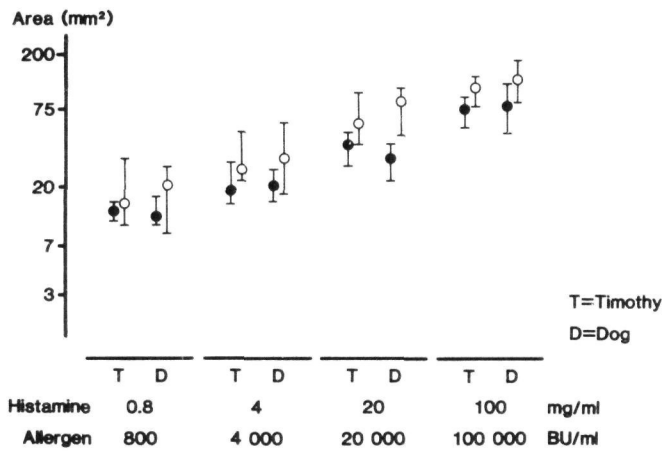


Fig. 2. The median and the 95 % confidence limits for the median of the four replicate weal areas obtained with each concentration of histamine (filled circles) and allergen (open circles) in the second series of experiments. T denotes timothy and D dog group.

15 mm² constitutes a lower limit for good precision with the SPT method, as claimed by Taudorf et al. (15), the class 10–20 mm² was divided into two sub-classes 10–15 and 15–20 mm².

The difference in slope between materials was tested by Student's *t* test (two-tailed) using paired data.

The slope of the dose response relationship calculated on the basis of weal areas, has a numerical value twice that obtained based on mean weal diameters.

The trials were approved by the local ethics committees and the patients gave their verbal informed consent.

RESULTS

Weal response to allergen and histamine

In the second series of experiments, a positive mean weal response was induced by histamine HCl, 0.8 mg/ml and higher concentrations (Fig. 1a and b). In all these patients the geometric mean of weal areas for the four tests with the same concentration of histamine was more than 7 mm², corresponding to 3 mm in diameter. Eleven patients of 20 (55 %) showed both positive and negative tests (< 7 mm²) to the same concentration of histamine, mainly 0.8 mg/ml. Seven single negative tests to his-

mine 0.8 mg/ml (7/80 tests, 9 %), in seven patients (7/20, 35 %) were recognized. The response to histamine 1 and 10 mg/ml was estimated to be on an average 15 and 35 mm², i.e. approximately 4.5 and 5.5 mm in diameter.

There was a considerable individual variation in sensitivity to the allergens. A mean weal area > 7 mm² was induced by 160 BU/ml of timothy and dog allergen preparations in five patients, in 19 by 800 BU/ml and in all 20 patients by 4000 BU/ml. Eighteen of 20 patients (90 %) showed both positive and negative test results to at least one concentration of allergen. Five patients of 20, gave both negative and positive responses to two concentrations. There were four negative single skin prick tests to 4000 BU/ml (Fig. 1c and d). The response to histamine varied less between patients than that to allergen. The difference in variation of the response to allergen and histamine implies that the 95 % confidence limits for allergen are wider than those for histamine (Fig. 2).

The response to dog and timothy allergen was slightly less than that of histamine HCl within the concentration range 0.8–100 mg/ml corresponding to 800–100,000 BU/ml (Figs. 1 and 2). However, a statistically significant difference was only found for dog allergen 20,000 BU/ml versus histamine HCl 20 mg/ml.

Precision of SPT

In the first experiment the precision (c.v.) of the SPT method was bad, about 50–70 % for timothy allergen preparation and histamine, at response levels < 5 mm in diameter (20 mm², using the area of the weal). The precision seemed to be slightly better for the histamine releasing substances.

In the second series of experiments the c.v. was higher at response levels <, than > 10 mm² (corresponding to 3.5 mm in diameter). This was due to the inclusion of weal reactions < 3 mm² (2 mm in diameter) (Fig. 3a) regarded as negative. The c.v. was similar for histamine and the two allergens respectively. Therefore the results obtained with timothy and dog were pooled as well as results obtained with histamine (Fig. 3b). There was no essential difference in c.v. for allergen and histamine for

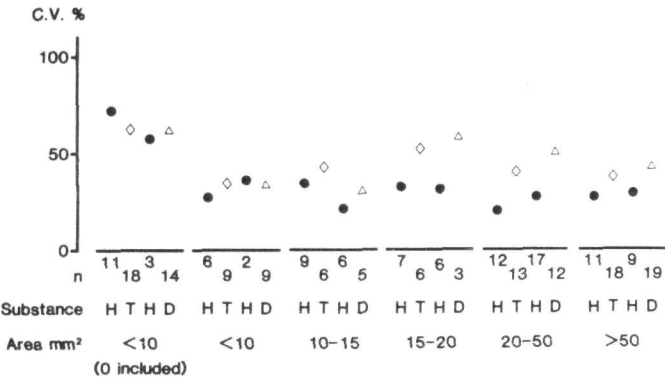


Fig. 3a. The c.v. for histamine (H), timothy (T) and dog allergen (D) for different mean weal diameters in the second series of experiments. To the left are indicated data obtained when negative responses (weal areas < 3 mm²) were included in the calculations.

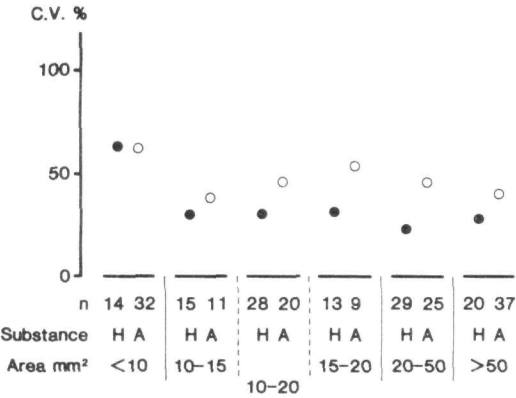


Fig. 3b. The c.v. for allergen (A) and histamine (H) responses. All data from the second series of experiments were pooled. Data obtained from the weal area interval 10-20 mm² are given separately and divided into two intervals 10-15 and 15-20 mm². n indicates the number of quadruplicate tests with the same concentration included in the respective classes, based on the geometric mean of quadruplicate tests.

level classes > 10 mm², but a tendency to lower c.v. for histamine than for allergen. A statistically significant difference between allergen and histamine was only found for one level class (20-50 mm²) (Fig. 3b).

Slope (b) of the dose response relationship

The median and 95 % confidence interval for the calculated slopes (b) for all substances in experiment I are given in Fig. 4a. The slopes were similar for the four tested substances and of the same order as previously reported for

Substance	n	Slope (b)				
		0.15	0.20	0.25	0.30	0.35
Histamine	5	M				
Timothy	7	M				
Anti-IgE	6	M				
Compound 48/80	4	M				

Fig. 4a. The slope for histamine, timothy, rabbit anti-human-IgE, and compound 48/80 from experiment I. The median and the 95 % confidence limits for the slope (b) calculated according to the model $\log D = a + b \log (\text{conc})$ are given. n indicates the number of patients with statistically approved data.

Substance	n	b		
		0.3	0.4	0.5
Dog	10	M		
Histamine	10	M		
Timothy	10	M		
Histamine	10	M		

Fig. 4b. The slope for timothy and dog allergen and histamine. The median and 95 % confidence limits are given. Differences given in the text were based on arithmetic means.

Substance	n	Range	b		
			0.3	0.4	0.5
Dog	9	Low	M		
		High	M		
Timothy	10	Low	M		
		High	M		
Histamine	10	Low	M		
		High	M		

Fig. 4c. The slope for the three highest concentrations (high) and the lowest of these and the preceding two lower concentrations (low). The median and 95 % confidence limits are given.

allergens (b = 0.2) (4). The geometric means of all responses in all patients to each concentration are plotted in Fig. 5. The individual regression lines were parallel and therefore data were pooled to give one dose response relationship for each substance.

The regression lines were found to be best fitted to the model $\log A = a + b (\text{conc})$ (data not given). The correlation coefficients (r) of the allergen dose response curves were > 0.97 in all but one of 20 patients.

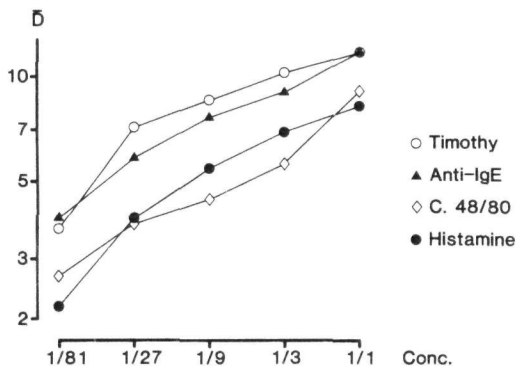


Fig. 5. The plotted "slopes" for all four substances used in experiment I, based on the geometric mean of all responses (two in each patient) to the concentrations tested.

The dose response curves for timothy and histamine had mean slopes of 0.40 (SD 0.12) and 0.38 (SD 0.04), respectively. There was no significant difference between the mean slopes (Fig. 4b), but a tendency towards steeper slopes for timothy. The mean slopes for the dog and histamine dose response relationships were 0.42 (SD 0.08) and 0.36 (SD 0.05) respectively (Fig. 4b). The mean slope of dog allergen was significantly steeper than the corresponding mean slope for histamine ($P < 0.05$). In four of 10 cases the dose response curves were not parallel. In three of them the allergen dose response curve was steeper and in the fourth the histamine. Thus, there was a tendency towards steeper slopes for both allergens than for histamine. The slope of allergen for the three highest concentrations used (500,000, 100,000 and 20,000 BU/ml) and the third to fifth concentration (20,000, 4000 and 800 BU/ml) of each substance were also found to be parallel (Fig. 4c). The same applied to histamine (100, 20, and 4 mg/ml and 4, 0.8, and 0.16 mg/ml, respectively). However, there was a tendency towards steeper slopes within the lower dose range.

DISCUSSION

In earlier studies (2, 3) and when the precision of the skin prick test method was investigated at different response levels, we regarded a weal diameter of 2 mm (3 mm²) to be the lowest positive response. However, for the investigation of the dose response relationship in experi-

ment II, we used a weal diameter of 3 mm (7 mm²) as a lower limit for positivity, because of the low precision of the skin prick test method with smaller weals.

It is important to use a relevant limit for positivity even in routine diagnostic work. Our results indicate that single true negative results, i.e. weals with a diameter < 2 mm, are sometimes obtained at low response levels. We therefore propose that 7 mm² or 3 mm in diameter is used as a practical lower limit for positivity in diagnostic work using the SPT method. We have also demonstrated that even if this cut off limit is used, many patients react with both positive and negative tests to the same concentration (Fig. 1 a-d). Thus duplicate tests should be recommended when screening for sensitization to avoid negative results.

Occasional negative responses were obtained with histamine HCl, 0.8 mg/ml, indicating that higher concentrations are more suitable as reference substance in SPT. Hordle et al. (7) found that the dose response relationship of allergens was linear only for weals $> 6-7$ mm², which supports our data. The concentration most suitable for routine diagnostic work will be discussed below.

The response to histamine varies little between patients when tests are performed by the same investigator (Fig. 1, 2). The response to allergen, on the other hand, varies widely as has previously been reported (3, 4). It has been proposed to relate the allergen response to the histamine response, e.g. to give the allergen weal size in % of the histamine weal size to correct the results for differences in SPT technique (1).

The weal response to dog and timothy allergen of the same labelled biological activity was similar, and histamine 0.8-100 mg/ml gave the same response as the corresponding allergen concentrations. These data show the relevance of the idea of biological standardization and the system used for the standardization of these allergens.

The precision of the SPT is essential when using SPT methods for different purposes, i.e. biological standardization, estimation of the relative potency of allergen extracts/preparations,

estimation of the difference in skin sensitivity during immunotherapy and other therapeutic measures, e.g. antihistamine therapy. As the area was used instead of the diameter in the second series of experiments, the coefficient of variation should be twice that of the first series of experiments. The response was not precise at low response levels, i.e. $<$ approx. 3.5 mm in diameter or 10 mm². Our group (4), Malling (9) and Taudorf et al. (15) all found a poor precision of the SPT method at low response levels. Based on these results, the Committee on Allergen Standardization within the Scandinavian Association of Allergology and Clinical Immunology has recommended the use of histamine 10 mg/ml (rather than 1 mg/ml) as reference substance in biological standardization. Our present results indicate that the reason for the poor precision at low response levels is the presence of negative reactions. In routine it seems reasonable to use this concentration of histamine if single SPTs are done, but 1 mg/ml can also be used if duplicate tests are performed and if an SPT technique is used that induces histamine weal areas $>$ 10 mm² (3.5 mm in diameter).

The low response to histamine 1 mg/ml reported here and earlier (3, 4, 9) may explain why many patients had to be excluded for statistical reasons from the final calculations in some previous BS trials (2, 3). The methods used included allergen concentrations eliciting weals smaller than those of histamine HCl 1 mg/ml in many patients. Occasional negative responses are common and therefore the precision is bad with such low concentrations. It is therefore essential to use sufficiently high concentrations of histamine and allergen in BS to induce positive weal responses in all patients tested. The sensitivity to allergens varies greatly between patients. Therefore the allergen concentrations used for BS should be chosen individually (Fig. 1c and d), to secure positive test results in all patients included in such trials on the basis of clinical sensitivity.

The SPT method used in routine should be sensitive, to diagnose all patients with clinical symptoms. In the present study all patients reacted with at least one positive weal response

($>$ 3 mm in diameter or 7 mm²) to 20,000 BU/ml. Differences in SPT technique may necessitate higher concentrations in some clinics than in others in order to identify all patients with clinical symptoms. Petterson et al. (14) recommended a concentration higher than 50,000 BU/ml for routine use. The reactivity in a population of patients allergic to the allergen in question varies over four powers of 10 (2, 3, 4). By definition the median sensitive patient gets a weal of the same size as histamine 1 mg/ml when tested with 1000 BU/ml, while the least sensitive patient included in the BS of an allergen would then need 100,000 BU/ml to get a weal of that size. As mentioned, many investigators get weals 3–4 mm in diameter i.e. close to the “cut off” limit of 3 mm, when using histamine 1 mg/ml. A routine diagnostic method like the SPT method should be sensitive. Thus 100,000 BU/ml seems to be a suitable concentration for SPT. This has been confirmed in a large study using this concentration (8).

We found that the dose response curves of the two allergens and of these allergens and histamine were not significantly different. The slopes did not differ significantly between the lower and upper part of the dose response curve for dog, timothy and histamine. However, the 95% confidence limits were wide and there was a tendency towards flatter slopes with histamine. There was also a tendency towards steeper slopes at the lower part of the dose response curve for both allergen and histamine. There might exist a small difference between the slopes mentioned. However, we failed to reject the null hypothesis due to the low number of patients investigated.

Malling (9) has claimed that there is no correlation between the increase of the histamine- and allergen-induced weal areas. In this study he only investigated histamine 1 and 10 mg/ml but a broader range of concentrations of *Cladosporium* allergen. *Cladosporium* showed a steeper slope in our experiments, which might explain the findings of Malling (9).

Dirksen et al. (5) have recently published data indicating that the dose response relationship of allergen is not best fitted to a log/log but

to a lin/log model. Turkeltaub et al. (16) have earlier stated that this is true for the sum of erythema diameters when using the intracutaneous test technique. However, Dirksen and Malling only used two points on the allergen and histamine dose response curves, respectively. Therefore we consider our results, showing parallelism in most cases between the dose responses for allergens and histamine over a wide range of concentrations, to be more reliable than those of Dirksen, and the finding of Turkeltaub et al. is only true for the sum of erythema diameters at low response levels.

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