

Quantitative skin prick testing

Dose-response of histamine- and allergen-induced wheal reactions

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Dose-response curves of histamine- and allergen-induced wheal areas were evaluated in seven normals (defined as negative skin prick test (SPT) to inhalant allergens and no clinical signs of allergy), seven latent allergics (positive SPT without allergic symptoms), and 20 manifest allergics (positive SPT and allergic symptoms). Three concentrations of histamine HCl (1, 10 and 100 mg/ml) and three 10-fold concentrations of nine inhalant allergens (birch, timothy, mugwort, horse, dog, cat, house dust mite, *Cladosporium* and *Alternaria*) in concentrations 1,000 10,000 and 100,000 BU/ml were used and linear regression was performed on the skin reactions. Only tests with an SD% < 40%, a log slope > 0.1, and a correlation coefficient > 0.95 were accepted. In normals a significantly higher concentration of histamine was needed to elicit a wheal reaction of 2 mm² (end-point) compared with allergics. Likewise, normals had a significantly higher slope i.e. steeper dose-response curve of histamine than manifest allergics. The slope of the allergen-induced wheal area was significantly higher than the histamine slope. No relation between corresponding slope of histamine and allergens was found ($Rho = 0.15$). The skin sensitivity equivalent to histamine calculated as the allergen concentration eliciting a wheal equal to histamine showed a median increase of 5-6 fold in allergen concentration by a 10-fold increase of histamine concentration. The highest correlation between the wheal area of a single allergen concentration and the skin sensitivity was found for allergen concentration 100,000 BU. Based on these results it is recommended that biological standardization of allergen extracts is based on quantitative skin titration of at least three 10-fold concentrations of both histamine and allergens and the biological activity (HEP unit) re-defined from histamine HCl 10 mg/ml.

Key words: biological standardization; quantitative skin test; skin prick test, skin sensitivity.

Accepted for publication 1 December 1986

CLINICAL ASPECTS

The advantages of quantitative skin test in biological standardization of allergen extracts have been accepted by standardization authorities. The relation between skin sensitivity (allergen concentration eliciting a wheal equal to histamine) estimated by histamine HCl 1, 10 and 100 mg/ml showed no constancy when using the actual histamine wheal area. Performing linear regression, however, on three 10-fold histamine concentrations and use of the estimated histamine area resulted in a highly significant correlation. To improve the biological standardization of allergens, quantitative skin testing with three 10-fold concentrations of both histamine and allergens is recommended. Furthermore, the biological potency should be expressed as the allergen concentration eliciting a wheal equal to the histamine HCl 10 mg/ml wheal area calculated from the dose-response curve.

Dose-response curves of histamine- and allergen-induced wheal reactions using skin prick test (SPT) have not been intensively studied. A

previous study (8) using only two concentrations of histamine showed no relation between corresponding slope of histamine and allergen

wheal area increase. The existence of parallel dose-response curves has been claimed by some investigators for wheal areas $> 20 \text{ mm}^2$ (5). In the first methodological study of standardized quantitative skin test Turkeltaub et al. (15) found a significantly steeper slope for short ragweed than for histamine, using the sum of erythema diameters in intradermal test.

Dose-response curves are not essential when SPT is used for diagnostic purposes but is crucial in biological standardization and in evaluation of changes in skin sensitivity initiated by immunotherapy or medical treatment. By definition, biological standardization has been performed with histamine dihydrochloride 1 mg/ml as positive reference (the HEP-system) (17). The positive reference has been changed to histamine dihydrochloride 10 mg/ml as proposed by the standardization committee at the XV Nordic Congress in Allergology, Turku, Finland 1984. However, the term HEP still refers to histamine HCl 1 mg/ml. This problem has been solved by performing biological standardization with histamine HCl 10 mg/ml and interpolating the result to histamine 1 mg/ml (5).

Most studies of quantitative SPT dose-response are performed with only one or few allergen extracts. The present study evaluates the dose-response curves of both histamine and allergens including a wide spectrum of pollens, animal dander, house dust mite and moulds. Furthermore, the relation between "HEP" estimated by histamine HCl 1, 10 and 100 mg/ml was estimated as well as the relation between skin sensitivity and the wheal area elicited by different allergen concentrations.

MATERIAL AND METHODS

Patients

A total of 34 subjects were tested: seven normals (3 females, 4 males, median age 36 years, range 31–37) defined as persons without allergic symptoms and negative SPT to standard inhalation allergens, seven latent allergics (4 females, 3 males, median age 34 years, range 26–37) defined as persons with positive SPT reac-

tion to one or more allergens but without clinical allergic symptoms, and 20 manifest allergics (6 females, 14 males, median age 31 years, range 19–51), i.e. patients with positive SPT and a case history of allergic diseases.

Excluded from the study were patients with eczema on the test area, patients treated with antihistamines, oral steroids $> 10 \text{ mg}$ prednisolone or topical steroids less than 72 h prior to testing and, finally, patients treated with immunotherapy within the last year (12). Informed consent to participate was obtained and the study was approved by the local Ethics Committee.

Test extracts

Pharmalgen® partially purified and biologically standardized lyophilized extract of birch (*Betula verrucosa*), timothy (*Phleum pratense*), mugwort (*Artemisia vulgaris*), horse, dog and cat dander, house dust mite (*Dermatophagoides pteronyssinus*), *Cladosporium herbarum* and *Alternaria iridis* were obtained from Pharmacia, Uppsala, Sweden. Only one batch of each allergen was used in all patients. The extracts were reconstituted in glycerol diluent (glycerol 50%, phenol 5 mg/ml, water for injection) in three concentrations (1,000, 10,000 and 100,000 BU/ml respectively). Positive references were histamine dihydrochloride 1, 10 and 100 mg/ml in glycerol diluent. The extracts were stored at 2–8°C when not in use, and the testing was completed within a 2-month period.

Skin test procedure

Quantitative SPT was performed as previously described (8, 9, 10). In short, duplicate determinations of histamine and allergens in three 10-fold concentrations were carried out in three straight rows on both forearms. The tests were spaced 2 cm apart and randomized to different sites. The skin reactions were recorded at maximum size (12–15 min) and the wheal area calculated by planimetry.

The reproducibility of double determinations was evaluated as coefficient of variation = $\text{SD}/\text{mean area} \times 100\%$. The mean wheal area was

plotted in a log-log system as a function of the allergen concentration, and linear regression using the least square method was performed (area = intercept + slope × allergen concentration). From the regression line the allergen concentration eliciting a wheal equal to histamine was calculated and defined as the skin sensitivity (histamine equivalent) and termed SSH. The “actual” SSH was calculated from the actual histamine wheal areas and the “corrected” SSH based on the calculated histamine wheal areas after performing linear regression on the histamine reactions. Thus the “corrected” SSH is calculated from the best fitted line of both histamine and allergens respectively.

Acceptance of skin test

Because only double determinations were used and testing with three 10-fold concentrations, the following criteria for acceptance of a test sequence were used. Only tests with a positive dose-response, i.e. wheal area of the lower concentration smaller than that with the higher concentrations, were accepted. Furthermore, the wheal area of histamine 1 mg/ml and allergen concentration 1,000 BU had to be larger than 3 mm². Tests were accepted in the case of a SD % < 40 %, a log slope > 0.1 and a correlation coefficient > 0.95.

Statistics

Differences between groups were tested by Mann-Whitney U-test. Correlations were calculated by the least square method and Spearman’s test.

RESULTS

In all patients the histamine test fulfilled the inclusion criteria. Allergen tests in latent allergics were positive at the concentration 100,000 BU and in some cases at 10,000 BU. In the group of manifest allergics 20 patients were included and in 19 the allergen tests were accepted according to inclusion criteria. In 68 allergen tests (38 %) no reaction was elicited by

SPT. Forty-three tests (24 %) were not accepted either because of a coefficient of variation exceeding 40 % (2 %), a correlation coefficient < 0.95 (3 %) or wheal area < 3 mm² of allergen concentration 1,000 BU (19 %). The present results are based on 69 accepted SPTs of 112 positive (62 %).

Histamine dose-response

The median wheal areas of histamine tests are shown in Table 1. A significant difference between normals and manifest allergics was found for histamine 1 and 10 mg/ml as was also the case for the difference between latent allergics and manifest allergics for all three tested histamine concentrations. In the case of histamine 1 mg/ml latent allergics showed double the wheal area compared with normals and manifest allergics double that of latent allergics.

From the histamine dose-response curve the histamine concentration eliciting a wheal area of 2 mm² (equivalent to end-point titration) was calculated. The concentrations eliciting such a wheal area in normal persons, latent and manifest allergics would be 0.53 mg/ml, 0.15 mg/ml, and 0.006 mg/ml respectively. The differences between normals and manifest allergics and latent allergics versus manifest allergics were significant (*P* < 0.001 and *P* < 0.05 respectively).

The slope calculated from the linear regression of the dose-response curves is an expression of the wheal area increase as a function of a 10-fold

Table 1
Medial wheal area (mm²) of histamine HCl 1, 10, and 100 mg/ml in normals, latent and manifest allergics

	Histamine concentrations					
	1 mg/ml		10 mg/ml		100 mg/ml	
Normals	↑ NS	3	↑ NS	10	↑ NS	44
Latent allergics	↓ ↑ *	6	**	↓ ↑ **	14	**
Manifest allergics	↓	13	↓	25	↓	61

NS not significant
* *P* < 0.05
** *P* < 0.01

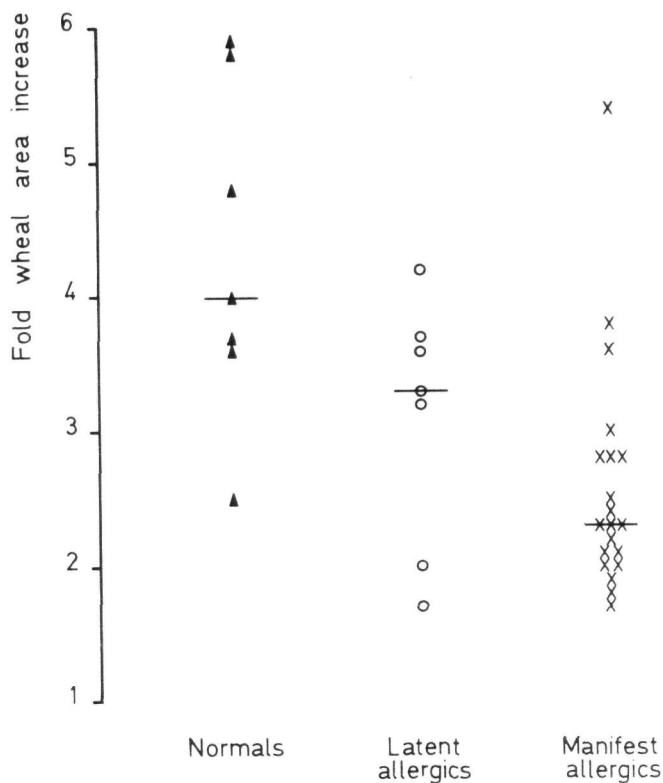


Fig. 1. The slope of dose-response curves of histamine HCl 1, 10 and 100 mg/ml in normals (▲), latent (○), and manifest (×) allergics. The slope is expressed as fold wheal area increase by 10-fold increase of histamine concentration.

extract increase. The slope is stated as fold wheal area increased by using 10 times more concentrated extract. The median wheal area increase of histamine in normals was 5-fold (range 2.5–5.9), in latent allergics 3.3-fold (range 1.7–4.2) and in manifest allergics 2.3-fold (range 1.7–3.8) (Fig. 1). Only the difference between normals and manifest allergics was significant ($P < 0.01$).

Allergen dose-response

From the total of 69 accepted tests 16 (23 %) were with pollen allergens, 26 (38 %) with animal dander, and 13 (19 %) with house dust mite and 14 (20 %) with moulds. The median wheal area increase (slope) was between 2.5-fold (mites) and 2.8 (pollen, animal dander and moulds) with a range of 1.5–6.9. No significant difference between the allergen groups was found (Fig. 2).

Relation between histamine and allergen dose-response

Corresponding wheal area increase (fold) of histamine and allergen skin tests is evident from Fig. 3. In the case of an identical dose-response curve = identical slope, the observations were expected to

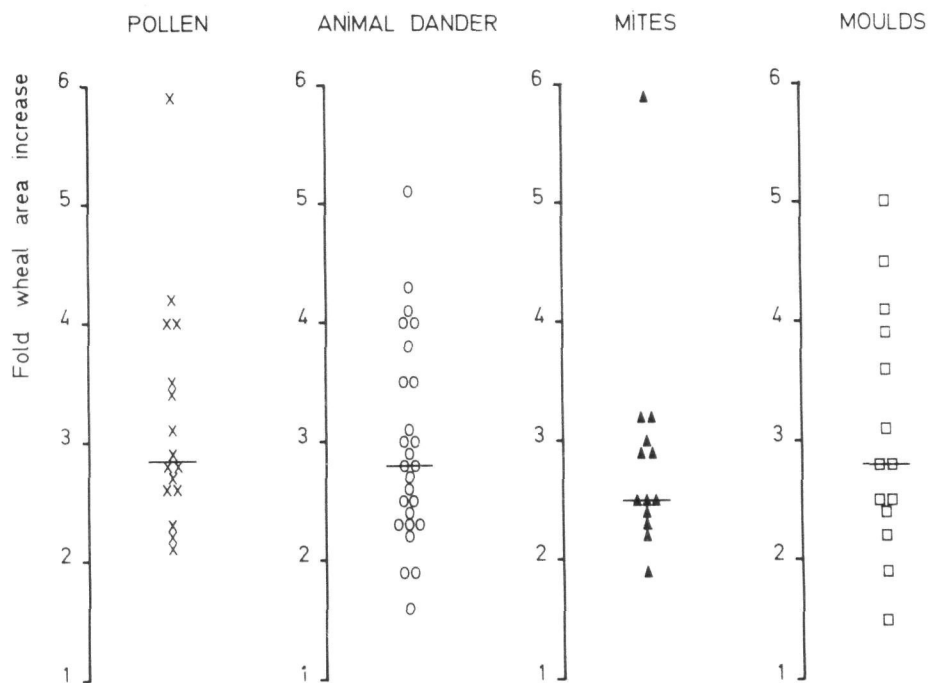


Fig. 2. The slope of allergen dose-response curves expressed in fold wheal area increase by 10-fold allergen increase. No significant difference within or between allergen groups was found. Pollen (×) (birch, timothy, mugwort), animal dander (○) (horse, dog, cat), house dust mite (▲), moulds (□) (*Cladosporium*, *Alternaria*).

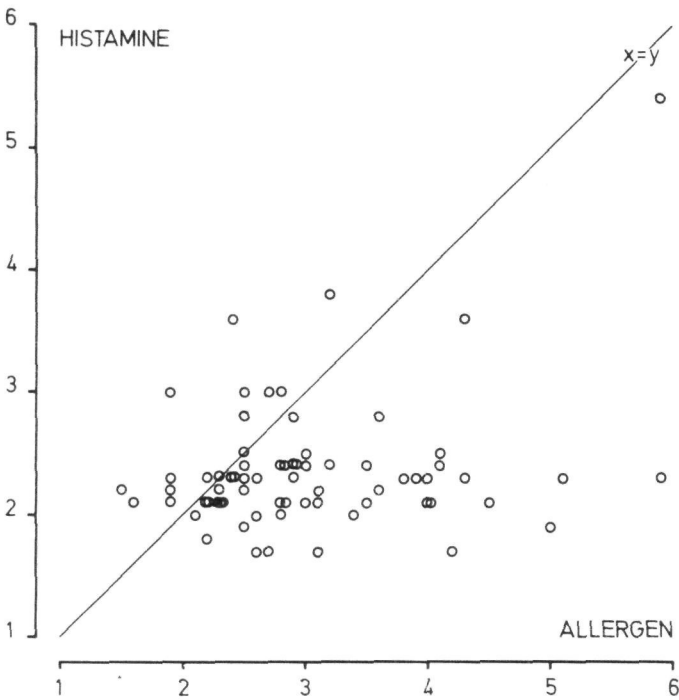


Fig. 3. Corresponding slope (fold wheal area increase) of histamine and allergen quantitative skin test. The line of identity ($x = y$) is marked. Calculation of the correlation coefficient (Spearman's $Rho = 0.15$) in 69 tests showed a non-significant relation.

be grouped along the line of identity ($x = y$). However, this was not the case. Eighty per cent of tests showed a higher slope of allergens than the corresponding histamine. Calculation of correlation showed a Spearman's Rho as low as 0.15 (NS).

To further evaluate the degree of parallelism between histamine and allergen dose-response, SPTs with a slope of allergen being within ± 0.5 fold of the histamine slope were accepted as parallel. Accordingly, parallelism was demonstrated in a total of 32 tests (46 %). Exceeding the limit to ± 1.0 -fold resulted in parallelism in 49 tests (70 %). An interesting interindividual difference in histamine and allergen parallelism was observed. Some patients with more than four accepted dose-response curves showed only one non-parallel test, whereas other patients had none or only one allergen slope parallel with histamine.

Skin sensitivity

The skin sensitivity equivalent to histamine (SSH) was calculated using both the "actual" wheal area of histamine 1, 10 and 100 mg/ml and

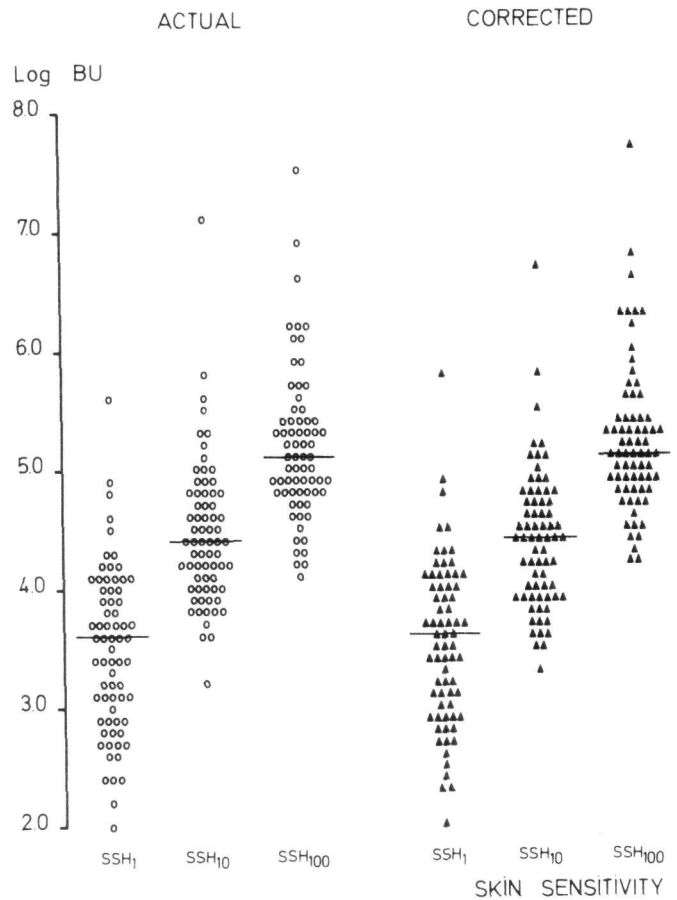


Fig. 4. Skin sensitivity estimated as the allergen concentration (log BU) eliciting a wheal equivalent to histamine HCl 1 mg/ml (SSH₁), 10 mg/ml (SSH₁₀), and 100 mg/ml (SSH₁₀₀). Skin sensitivity calculated from the "actual" wheal area (O) and from the "corrected" (▲) (after performing linear regression) histamine reaction.

the "corrected" area as stated in Material and Methods. Fig. 4 shows the results of these two estimates. The median SSH₁ was 4,000 BU, SSH₁₀ 25,000 BU, and SSH₁₀₀ 126,000 BU. No statistically significant difference between the "actual" SSH and "corrected" SSH was found.

Corresponding values of the difference between SSH₁ and SSH₁₀ versus SSH₁₀ and SSH₁₀₀ are shown in Fig. 5. Using the "actual" SSH (Fig. 5A) the median difference between SSH₁ and SSH₁₀ was 0.85 log BU (range 0.25–2.2), equivalent to a factor 7 (range 1.8–160). The corresponding values of the SSH₁₀ versus SSH₁₀₀ difference was 0.7 log BU (range 0.25–2.0), a factor 5 (range 1.8–100). No constancy in the relation between $\Delta SSH_1/SSH_{10}$ and $\Delta SSH_{10}/SSH_{100}$ was found, however, with a correlation coefficient of only 0.36.

The use of "corrected" SSH (Fig. 5B) shows the constant relation between SSH₁, SSH₁₀, and

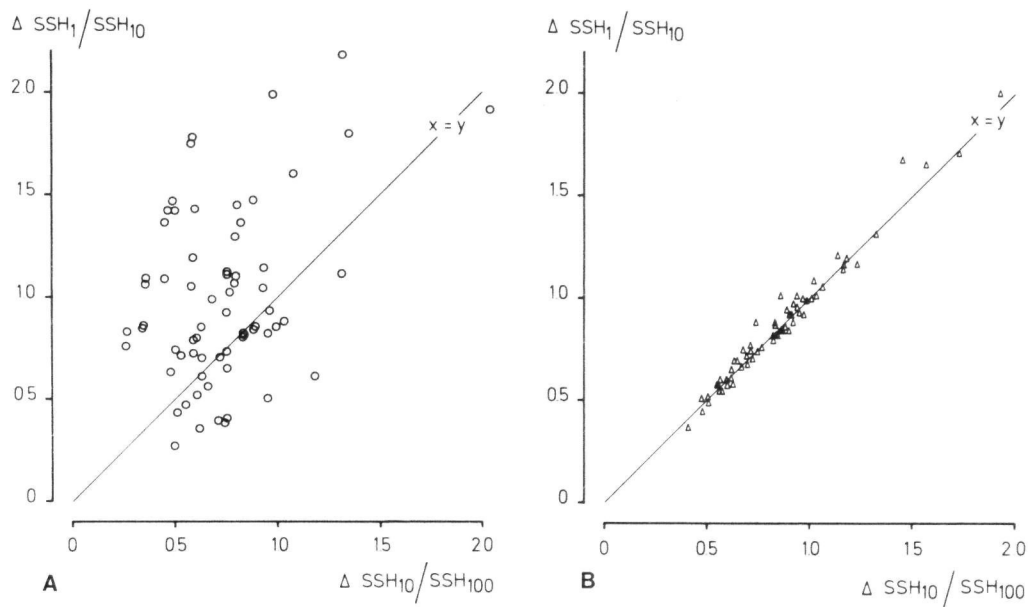


Fig. 5. Relation between difference (log BU) in skin sensitivity estimated by histamine 1 mg/ml and histamine 10 mg/ml ($\Delta \text{SSH}_1/\text{SSH}_{10}$) versus histamine 10 mg/ml and histamine 100 mg/ml ($\Delta \text{SSH}_{10}/\text{SSH}_{100}$) using the "actual" skin sensitivity ($\circ$) (5A), Spearman's Rho 0.21 (NS) and the "corrected" skin sensitivity (Δ) (5B), Spearman's Rho 0.98 ($P < 0.0001$).

SSH_{100} with a correlation coefficient of 0.99 and a slope of 0.94 not different from the line of identity. The median difference of SSH_1 versus SSH_{10} and SSH_{10} versus SSH_{100} is 0.8 (range 0.4–2.0), equivalent to a factor 6.3 (range 2.5–100).

Relation between skin sensitivity and wheal area

A highly significant inverse correlation between skin sensitivity and log wheal area was found (Fig. 6). The correlation coefficient was 0.74 and the slope -1.5 in the case of SSH_1 versus wheal area of allergen concentration 1,000 BU. The corresponding figures for SSH_{10} and 10,000 BU were $r = 0.82$, slope -1.7 and for SSH_{100} versus wheal area 100,000 BU $r = 0.85$ and slope -2.0 . Fig. 6 further shows that if a single allergen concentration is preferred rather than a skin test titration, the highest approximation to an expression of skin sensitivity is obtained by an allergen concentration of 100,000 BU. Skin sensitivity (SSH) versus the absolute wheal area showed a hyperbolic curve response.

DISCUSSION

In this study the accuracy of the quantitative skin test carried out in a standardized manner

has shown to be equivalent to that of other tests used in the diagnosis of allergy (12). Only 5 % of tests with positive wheal reactions in all three concentrations were excluded due to a low reproducibility or non-linear dose-response. Because of its simplicity and inexpensiveness the skin test should be recommended a more pronounced role in the evaluation of the immunological mechanisms involved in allergy (2, 12, 13).

The differences observed in the histamine dose-response curves between normal persons, latent and manifest allergics have not been published previously. As shown in Table 1, normal persons produce a significantly smaller wheal by histamine 1 mg/ml compared to manifest allergics, an observation that also holds for histamine 10 mg/ml, although the relative difference in wheal area is less than that of histamine 1 mg/ml. The median wheal area of histamine 1 mg/ml in normal persons is about one fourth of the area in manifest allergics, with latent allergics presenting intermediate wheal areas. The reason for the non-significant difference between normals and latent allergics is probably a consequence of the relatively low number of test persons. The differences in skin reactivity between allergics and non-allergics

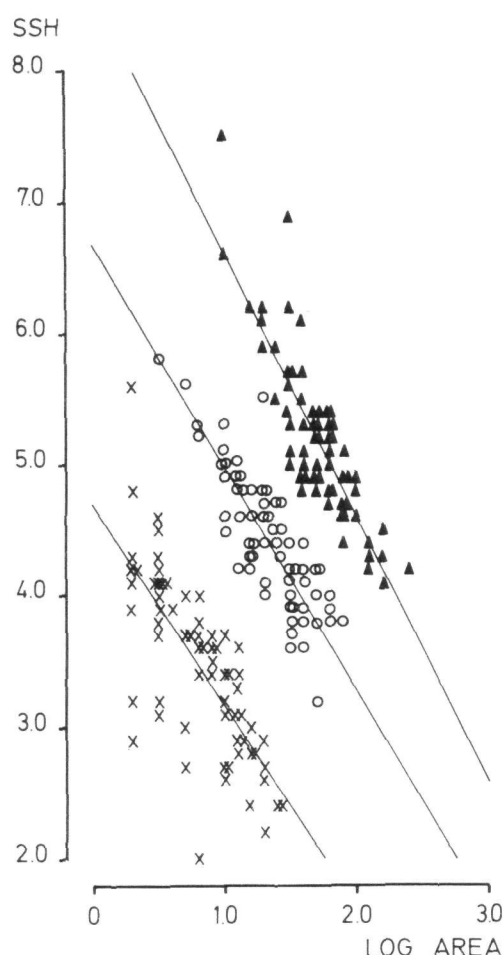


Fig. 6. Relation between wheal area of a single allergen concentration and corresponding skin sensitivity (allergen concentration eliciting a wheal equal to histamine). $\times$ = skin sensitivity histamine 1 mg/ml/allergen concentration 1,000 BU ($r = -0.74$). $\circ$ = skin sensitivity histamine 10 mg/ml/allergen concentration 10,000 BU ($r = -0.82$). $\blacktriangle$ = skin sensitivity histamine 100 mg/ml/allergen concentration 100,000 BU ($r = -0.85$).

have been observed by van Aspern (16), but only in infants at 4 months of age.

The observed difference in wheal area of the three histamine concentrations in normals and allergics is equivalent to the response in bronchial histamine challenge. Asthmatics have a significantly lower threshold in relation to normals, estimated by the histamine or methacholine concentration inducing a 20% decrease in FEV_1 (PC_{20}) (3, 6, 13). In skin test the end-point (calculated from the dose-response curve as the histamine concentration eliciting a wheal of 2 mm²) representing the break of the curve might be compared to the PC_{20} in bronchial challenge. The present data show that

equivalent to bronchial challenge (14), allergics are significantly more sensitive to histamine in the skin compared with normals.

The steepness (slope) of the histamine dose-response curve (Fig. 1) expresses a significantly lower slope (flatter dose-response curve) for allergics compared with normals. Unfortunately, this phenomenon cannot be compared to the dose-response of bronchial histamine challenge as the latter has to be terminated before a full range dose-response is obtained (increasing the histamine concentration to obtain a further decrease in FEV_1 of more than 20%, i.e. to 50–70%, might be dangerous to the patient and thus unethical). However, it could readily be assumed that although allergics are more sensitive to histamine than normals, estimated by the minimal concentration eliciting a response, a further increase of the histamine concentration results in a considerably lower decrease in FEV_1 in allergics than in normals. This might be caused by a lower number of histamine receptors in normals than in allergics or an imbalance in the number of H_1 - and H_2 -receptors (11), while the responsiveness of the histamine receptors in allergics is reduced by the more or less constant exposure to histamine (tachyphylaxis) (3).

The observed differences between normals, latent allergics and manifest allergics in histamine dose-response might be useful as a prognostic test, but has to be confirmed in larger groups of patients. The slope of allergen-induced wheal area increase was in accordance with those previously published with mould extracts (9). The interindividual variation was considerable, but no difference was found between different inhalant allergens (Fig. 2). The slope of allergen-induced wheal area increase, however, was significantly higher than the histamine slope, and the present study was not capable of demonstrating parallelism between histamine and allergen dose-response curve. This is in accordance with previously published data (8, 15) but in contrast to the findings of Dreborg et al. (5). The non-parallel dose-response curve of histamine and allergens and the considerable intraindividual variation in response to different allergens might be caused by several factors. The releasability of

histamine from mast cells by allergens might vary, i.e. increasing the allergen dose by a factor 10 often results in a more than 10-fold increase in histamine release from mast cells. This hypothesis is supported by the findings in *in vitro* histamine release from circulating basophil granulocytes (7). Other mediators involved in the allergen-induced wheal response might of course be responsible for this phenomenon (4). The lack of parallelism, however, implies that biological standardization in relation to the HEP term has to be performed with histamine 1 mg/ml or, as earlier pointed out, redefined from histamine 10 mg/ml because of the higher reproducibility of this concentration (8).

Considering the advantage of the quantitative skin test and the histamine equivalent skin sensitivity (9, 10), the use of one histamine concentration would seem irrational as testing with several 10-fold histamine and allergen concentrations and performance of linear regression provide a much more exact determination of the skin sensitivity. As shown in Fig. 5B the use of this "corrected" skin sensitivity results in a constant ratio of skin sensitivity estimated by histamine 1 mg/ml versus 10 mg/ml or 10 mg/ml versus 100 mg/ml in spite of histamine and allergen dose-responses being non-parallel. This is in contrast to the results obtained using "single" histamine concentrations (the "actual" skin sensitivity (Fig. 5A)). With this approach and the strict criteria for acceptance of a skin test titration as in this study, a very accurate skin test can be performed, elucidating even minor variations in skin sensitivity (evaluation of the antiallergic capacity of new pharmaka).

Quantitative skin test is not convenient for routine screening of IgE skin sensitivity (1, 5). The relation between the absolute wheal area and the corresponding skin sensitivity was evaluated for allergen concentrations 1,000, 10,000 and 100,000 BU. A highly inverse correlation was found for all three allergen concentrations. The highest correlation or the least deviation from a straight line was found for the allergen concentration 100,000 BU, indicating that this concentration represents the best alternative if only a single skin test concentration is employed to estimate the skin sensitivity.

From these data it can be concluded that the existence of non-parallelism between histamine and allergen dose-response curves must imply that biological standardization using the histamine equivalent reaction should be performed with both histamine and allergen titrations. The histamine concentration of 10 mg/ml (8) is superior in reproducibility to 1 mg/ml and is therefore recommended as the positive reference. Not the actual but the corrected histamine 10 mg/ml wheal area from a dose-response curve of histamine 1, 10 and 100 mg/ml should be used. For routine screening the highest correlation between the wheal area and the estimated sensitivity was obtained using allergen concentration 100,000 BU.

ACKNOWLEDGEMENT

Histamine and allergen extracts were kindly supplied by Pharmacia A/S, Hillerød, Denmark.

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