

Diagnosis and Immunotherapy of Mould Allergy

II. Reproducibility and Relationship between Skin Sensitivity Estimated by End-Point Titration and Histamine Equivalent Reaction Using Skin Prick Test and Intradermal Test

H.-J. MALLING

Laboratory of Clinical Allergology, State University Hospital, Copenhagen, Denmark

To determine reproducibility and the optimal way of expressing skin sensitivity, simultaneous skin prick tests (SPT) and intradermal tests (ICT) were performed on 25 mould-allergic patients. The patients had a well-documented history of allergy to *Cladosporium* and *Alternaria* and were tested with partially purified standardized extracts of these two mould species. Skin prick tests were carried out on the volar side of the forearm and intradermal tests on the backs of the patients. The skin tests were performed as titration using quadruplicate determinations of 10-fold allergen dilutions. The area of the skin reactions measured by planimetry were plotted in a log-log system as a function of the allergen concentration. The reproducibility (SD/mean area $\times$ 100%) of the ICTs was significantly higher than that of the SPTs (17% versus 29%). A very low reproducibility was found with wheal areas $< 5 \text{ mm}^2$. The dose response curve of the SPT wheal area was steeper than that obtained with ICT, both concerning ICT wheal and flare. Increasing the allergen concentration with a factor 10 resulted in a doubling of the wheal area in SPT, in contrast to a factor 1.7 using ICT. The coefficient of correlation using linear regression on the dose response curve was always higher than 0.9 with SPT and ICT wheal, but significantly lower with ICT flare. Skin sensitivity was estimated as end-point and histamine equivalent reaction. No significant correlation between SPT and ICT end-point titration was found contrary to the histamine equivalent reaction. The difference in allergen concentration between end-point and histamine equivalent reaction was a factor 500 (median) for both SPT and ICT. Due to the lower reproducibility of small skin reactions, the histamine equivalent reaction is to be preferred as an estimate of skin sensitivity. The simplicity and the steeper dose response curve of SPT outweigh the lower reproducibility compared with ICT, and with the use of potent allergen extracts, ICT has no advantages over SPT.

Key words: end-point titration; histamine equivalent reaction; intradermal test; mould allergy; reproducibility; skin prick test; skin sensitivity.

Accepted for publication 28 November 1984

CLINICAL ASPECTS

The optimal method of skin testing in allergic patients is a subject of discussion. Skin prick testing has advantages as it is simple and quick compared with intradermal testing. These advantages and the steeper dose-response outweigh the lower reproducibility of the skin prick test. The reproducibility of small skin reactions is low and therefore the larger histamine equivalent reaction is to be preferred to end-point as an expression of skin sensitivity. A standardized quantitative skin prick test titration seems to be the most suitable method for evaluating changes in skin sensitivity caused by various treatments.

For more than a century skin testing has been the foundation of diagnostic procedure in allergy (10). In spite of the recent introduction of *in vitro* techniques (radio allergosorbent test, crossed radio immunoelectrophoresis, etc.) the most common screening procedure is still the skin test.

Skin testing can be carried out by two different methods: 1) as an intracutaneous (ICT) or intradermal test in which the extract is injected into the superficial layer of the dermis (10, 12, 18), and 2) as a puncture or skin prick test (SPT) by which the extract is allowed to penetrate down into the dermis through some sort of channel (3, 10, 12).

The advantages, correlation and reproducibility of ICT and SPT have been compared in several studies (4, 8, 11). In the evaluation of skin testing, two factors are of principal importance: the reproducibility and a meaningful expression of the degree of sensitivity. A simultaneous study of SPT and ICT using mould allergens has been carried out to investigate these parameters.

MATERIAL AND METHODS

Patients

Twenty-five adult asthmatics were tested (median age 24, range 17–45 years; 9 females, 16 males). All patients had an unequivocal clinical history of mould-induced asthma. The diagnosis was further based on positive SPT, RAST and bronchial challenge using the same batch of extracts. Thirteen were allergic to *Cladosporium* and 12 to *Alternaria*.

Patients with eczema at the test area, patients treated with antihistamines, oral steroids >10 mg prednisolone a day or patients using topical steroids 72 h prior to testing, and, finally, patients treated with immunotherapy within the last 12 months were excluded from the study. Informed consent to participate was obtained and the study was approved by the local ethics committee.

Test extracts

Partially purified and standardized lyophilized extracts of *Cladosporium* and *Alternaria* (Pharmalgen®) were obtained from Pharmacia, Uppsala, Sweden. The extracts were reconstituted in HSA buffer (0.03% human albumin, 0.9% sodium chloride and 0.4% phenol) to a concentration of 1:20, 1:100 and 10-fold to 1:1,000,000 w/v. Extracts were reconstituted and diluted on the day of the skin test to assure maximum potency. The same batch was used in all patients.

Extracts, histamine and diluents were stored at 2–8°C when not in use. Positive references were histamine dihydrochloride 0.1, 1, and 10 mg/ml in albumin diluent, and as a negative control albumin diluent was used.

Skin test procedures

Intracutaneous tests were performed using 1 ml syringes and 26-gauge needles. A "micro papule" technique introducing a volume of approximately 0.02 ml in the superficial layer of the dermis resulting in a papule of 3 mm in diameter was used (12, 18). ICT was performed in quadruplicate on the backs of the patients. A separate randomizing scheme was used for all tests. Injection sites were spaced about 7 cm apart to prevent reaction overlap. The technique described by Norman (12) was used. All patients were tested with concentrations giving either a trace or negative reaction and until a reaction with flare >50 mm was elicited. The positive and negative controls in ICT were histamine HCl 0.1 mg/ml and HSA diluent, respectively.

Skin prick testing was carried out on the volar side of the forearms. Quadruplicate determinations of allergen dilutions 1:100,000 to 1:100, and 1:20 w/v, histamine HCl 1 and 10 mg/ml and diluent were performed simultaneously and with the same extracts as for ICT. The tests were spaced approximately 3 cm apart and randomized to different sites. SPT was performed with a 1 mm precision needle as previously described (7). After the prick test the extract

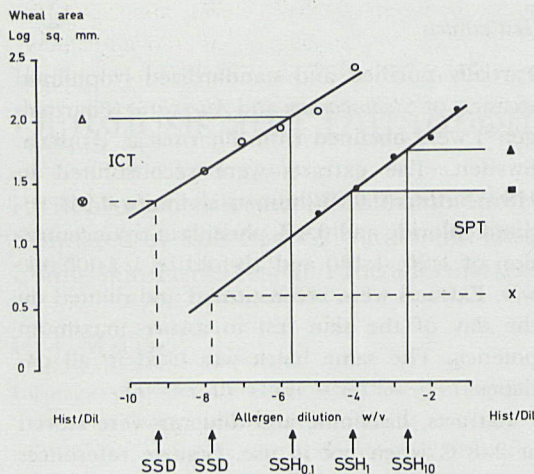


Fig. 1. Dose-response curve of skin test-induced wheal area as a function of allergen concentration in a log-log system. A linear regression is performed on the mean of quadruplicate determinations of five 10-fold allergen dilutions.

The allergen concentration eliciting a wheal equal in size to that obtained with histamine and diluent is estimated as an expression of skin sensitivity.

- allergen-induced wheal area, ICT.
- △ histamine hydrochloride 0.1 mg/ml-induced wheal, ICT.
- ⊗ diluent-induced wheal, ICT.
- allergen-induced wheal area, SPT.
- ▲ histamine hydrochloride 10 mg/ml-induced wheal area, SPT.
- histamine hydrochloride 1 mg/ml-induced wheal area, SPT.
- × diluent-induced wheal area, SPT.

SSH₁₀, SSH₁, SSH_{0.1} skin sensitivity equal to histamine hydrochloride 10 and 1 mg/ml in SPT and 0.1 mg/ml in ICT, respectively. SSD skin sensitivity equal to diluent (end-point titration).

drops were wiped off immediately (13).

ICT was performed by two experienced nurses and SPT by the author.

Recording of skin reactions

The skin reactions were recorded at maximum size, i.e. for histamine after approximately 12 min and for allergen extracts after 15 min. The contours of the wheal and flare were outlined with a fine fiber-tip pen and transferred to a record sheet by tape. The area of the wheal (SPT and ICT) and flare (ICT only) were measured by planimetry (Hewlett-Packard 9111 A

Graphics Tablet interfaced to a Hewlett-Packard 85).

Calculation of skin test results

The reproducibility of the quadruplicate skin test determinations was calculated as coefficient of variation = $SD/mean\ area \times 100\%$.

The mean skin area was plotted in a log-log system as a function of the allergen dilution, and a linear regression using the least square method was performed ($\log\ area = \log\ intercept + \log\ slope \times \log\ allergen\ dilution$).

From the regression line, the allergen concentration eliciting a skin reaction equal to the one produced by diluent was interpolated and defined as the end-point (skin sensitivity diluent, SSD). The histamine equivalent reaction (skin sensitivity histamine SSH₁₀, SSH₁, and SSH_{0.1}) regarding histamine dihydrochloride 10 mg/ml and 1 mg/ml (SPT) and 0.1 mg/ml (ICT) was calculated in the same way (Fig. 1).

Statistics

Parametric statistics were applied on normally distributed parameters, i.e. to estimate inter-individual reproducibility and to perform linear regression.

Non-parametric statistics were used to compare reproducibility and skin reactions obtained with the two techniques (Wilcoxon's signed rank test). Correlation between different estimates of sensitivity was calculated by Spearman's test. Non-paired data were tested by Mann-Whitney U-test.

RESULTS

Reproducibility

Corresponding values of wheal area and reproducibility of histamine-induced skin reactions are evident from Fig. 2. The median wheal area of histamine 10 mg/ml was 38 mm² (0.25 and 0.75 quantile 27–43 mm²). The corresponding reproducibility (SD%) was median 18% (9–27). The figures for histamine 1 mg/ml were wheal area 16 mm² (14–19) and SD% 27% (13–40).

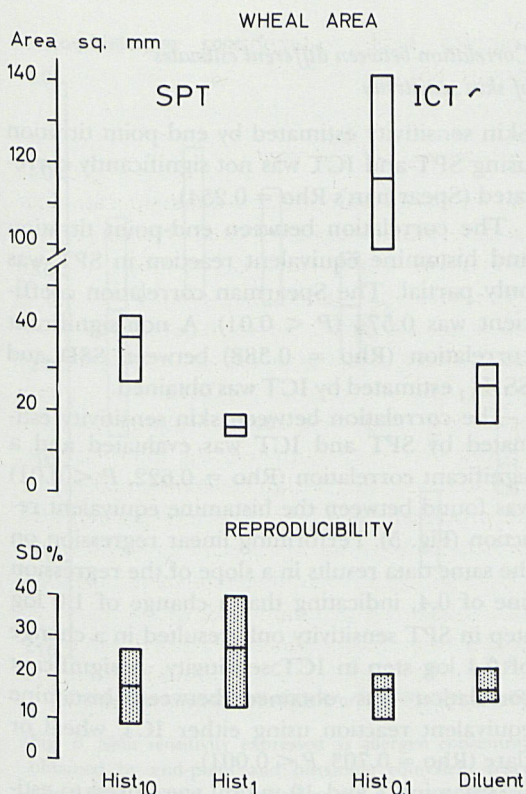


Fig. 2. Corresponding values of histamine-induced wheal area (open columns) and reproducibility (dotted columns) using SPT and ICT. Hist₁₀, Hist₁, and Hist_{0.1} represent histamine HCl 10, 1, and 0.1 mg/ml, respectively. The median and 0.25 to 0.75 quantile values are marked.

With ICT using histamine 0.1 mg/ml, the wheal area was 116 mm² (99–141) and SD% 17% (10–21).

The diluent reactions in SPT were negligible (<2 mm²). Regarding ICT the median wheal area was 26 mm² (17–31) and reproducibility 17% (14–22).

Reproducibility was significantly higher with histamine 10 mg/ml ($P < 0.02$) compared with histamine 1 mg/ml (SPT). No significant difference was found between histamine 10 mg/ml (SPT) and histamine 0.1 mg/ml (ICT).

The relation between the allergen-induced wheal area and reproducibility as a function of allergen concentration is shown in Fig. 3. Using the same allergen dilution the median wheal area with ICT is about 30 times larger than that with SPT. Generally, the reproducibility of ICT is higher than that of SPT with median SD%

values between 15–20% (ICT) and 25–43% (SPT). The lowest degree of reproducibility (highest SD%) in SPT was found with very small wheal areas (<5 mm²) of dilution 10⁻⁴ w/v. This difference compared with the other SPT allergen concentrations is significant ($P < 0.05$). The overall median reproducibility of allergen-induced wheals was SD% = 29% (SPT) of 93 quadruplicate determinations and 17% (ICT) of 108 quadruplicate tests. The difference is significant ($P < 0.01$). The reproducibility of corresponding allergen- and histamine-induced wheals and erythematous flare (Fig. 4) was estimated. The wheals induced by the allergen tended to be more reproducible than the flares (84 quadruplicate tests), but the difference was not significant ($P = 0.06$).

Dose response

Using linear regression the slope of the regression line is an expression of the wheal/flare

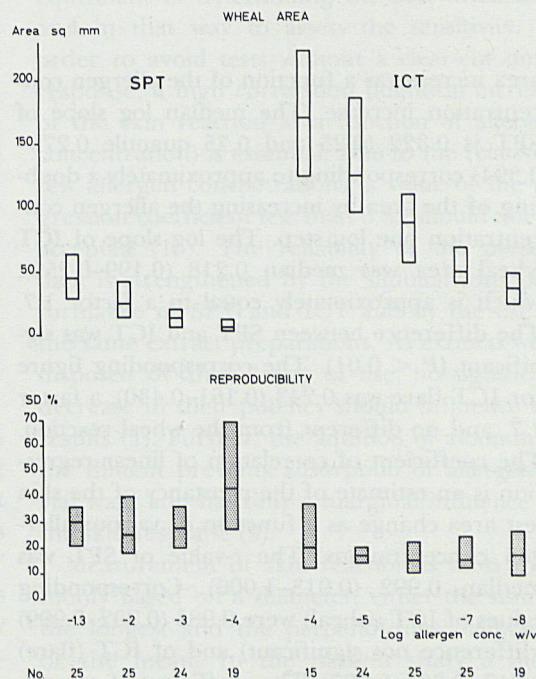


Fig. 3. Corresponding values of allergen-induced wheal area (open columns) and reproducibility (dotted columns) as a function of allergen concentration of SPT and ICT. The number of tests at each concentration is stated. Median and 0.25 to 0.75 quantile values are marked.

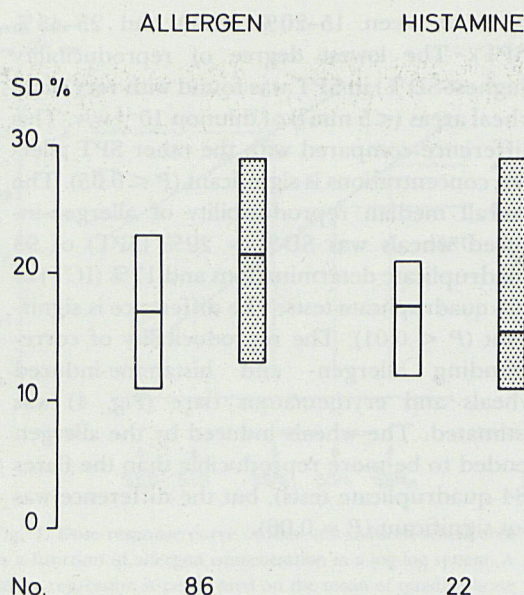


Fig. 4. The overall reproducibility of ICT wheal □ and flare ▤ reaction using allergen and histamine. The numbers stated represent the number of quadruplicate tests.

area increase as a function of the allergen concentration increase. The median log slope of SPT is 0.322 (0.25 and 0.75 quantile 0.270–0.394) corresponding to approximately a doubling of the area by increasing the allergen concentration one log step. The log slope of ICT wheal area was median 0.218 (0.199–0.259), which is approximately equal to a factor 1.7. The difference between SPT and ICT was significant ($P < 0.01$). The corresponding figure for ICT flare was 0.243 (0.161–0.430), a factor 1.7, and no different from the wheal reaction. The coefficient of correlation of linear regression is an estimate of the constancy of the skin test area change as a function of various allergen concentrations. The r -value of SPT was median 0.992 (0.913–1.000). Corresponding values of ICT (wheal) were 0.983 (0.902–0.999) (difference not significant) and of ICT (flare) 0.917 (0.801–0.997). The coefficient of correlation of the ICT flare is significantly ($P < 0.01$) lower compared with that of the wheal area.

Correlation between different estimates of skin sensitivity

Skin sensitivity estimated by end-point titration using SPT and ICT was not significantly correlated (Spearman's $Rho = 0.254$).

The correlation between end-point titration and histamine equivalent reaction in SPT was only partial. The Spearman correlation coefficient was 0.574 ($P < 0.01$). A non-significant correlation ($Rho = 0.388$) between SSD and $SSH_{0.1}$ estimated by ICT was obtained.

The correlation between skin sensitivity estimated by SPT and ICT was evaluated and a significant correlation ($Rho = 0.622$, $P < 0.01$) was found between the histamine equivalent reaction (Fig. 5). Performing linear regression on the same data results in a slope of the regression line of 0.4, indicating that a change of 1.0 log step in SPT sensitivity only resulted in a change of 0.4 log step in ICT sensitivity. A significant correlation was obtained between histamine equivalent reaction using either ICT wheal or flare ($Rho = 0.703$, $P < 0.001$).

Histamine 1 and 10 mg/ml were used to estimate the skin sensitivity in SPT. These two skin sensitivity estimates were significantly correlated ($Rho = 0.830$, $P < 0.001$). The slope of the linear regression line was 0.69.

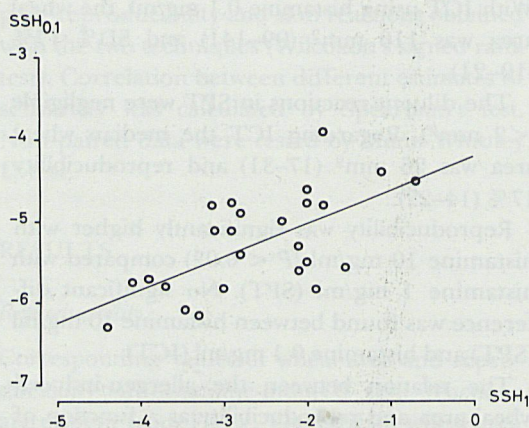


Fig. 5. Correlation between histamine equivalent skin sensitivity estimated by SPT (SSH_1) and ICT ($SSH_{0.1}$). Symbols as in Fig. 1.

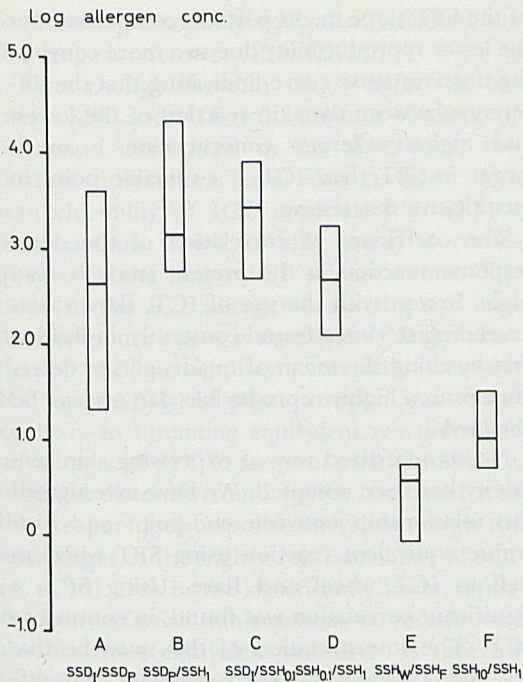


Fig. 6. Skin sensitivity expressed as allergen concentration obtained by end-point and histamine equivalent reaction using SPT and ICT. The median and 0.25 to 0.75 quantile differences are given.

SSD_I = end-point sensitivity, ICT; SSD_P = end-point sensitivity, SPT; SSH_{0.1} = histamine equivalent sensitivity, ICT; SSH₁ = histamine equivalent sensitivity SPT using histamine HCl 1 mg/ml; SSH₁₀ = histamine equivalent sensitivity SPT using histamine 10 mg/ml; SSH_W = histamine equivalent sensitivity ICT wheal reaction; SSH_F = histamine equivalent sensitivity ICT flare reaction.

Difference between various estimates of skin sensitivity

The difference in the allergen concentrations applied during the various ways of estimating skin sensitivity is evident from Fig. 6. End-point titration using ICT requires about 500 times (range 20–4,000) more diluted extract than SPT (median value). The corresponding figures comparing SPT end-point and histamine 1 mg/ml equivalent reaction, ICT end-point and histamine equivalent reaction and ICT versus SPT histamine (SPT histamine 1 mg/ml) reaction were 1,400; 2,600 and 500 times, respectively (range 600–21,000; 500–9,000; 120–1,700).

The difference in allergen concentration between ICT histamine wheal and flare was a factor 3 (range 1–6) and between SPT histamine 10 mg/ml and 1 mg/ml the figure is a factor 10 (range 5–30). The degree of correlation between the various estimates is also expressed by the range of the individual estimates (Fig. 6).

DISCUSSION

The choice of a definition of skin sensitivity is arbitrary and the most important factor is the reproducibility. The present study was designed in order to determine both the reproducibility and the optimal way of expressing skin sensitivity. Reproducibility was evaluated by the use of quadruplicate tests with both histamine and allergen extract. Skin sensitivity was estimated as both end-point (4, 12) and as histamine equivalent reaction (1, 2, 20). Quantitative skin testing (16) was chosen in order to obtain the advantages of all allergen concentrations being equivalent in determining the best fitted line, and in that way to assess the sensitivity. In order to avoid tests without a clear-cut dose-response, a high correlation (identical increase of the skin reaction as a function of allergen concentration) is essential. Due to the relatively few allergen concentrations, a value of the regression coefficient less than 0.85 should not be accepted (16). The reliability of the present data is strengthened by the simultaneous performance of SPT and ICT and by the use of the same extract preparations. As extracts were disposed of after 1 day of use, no significant decrease in their potency should influence the results (1). Further, the addition of albumin to the diluent prevents adsorption of allergen to vial walls and has only a marginal influence on the skin response (9).

Measurement of skin reaction is most commonly based on a diameter, either the sum of the longest and the perpendicular diameters, or the mean. In the present study a more accurate measurement was used. Computerized measurement of the skin reaction has shown to be more sensitive in revealing differences in area that could not be found by the use of

diameters (14), and planimetric measurements are more accurate (14, 15).

The reproducibility of skin tests depends among other things on how the area is measured. A coefficient of variation of the mean diameter of 20% as recommended by the Nordic Society of Allergology (2) corresponds to approximately 40% (mathematical calculation) using the area. This is extremely important in the comparison of different works. Intradermal tests are considered the most accurate (10, 12, 16, 17), but with the use of a standardized 2 mm Morrow Brown needle, almost the same degree of reliability as with intradermal tests was obtained in SPT (11). The coefficient of variation found in SPT is about 10–50% (3, 5, 7, 19). Tests with a coefficient of variation less than 40% (area) equivalent to 20% measured by diameter are acceptable (2). The present study has shown that both SPT and ICT fulfil these requirements provided the area of the skin reaction is more than 5 mm² (Fig. 3). Very large reactions obtained with ICT result in lower reproducibility, but in the range of 5 to about 150 mm² no dependency on the skin reaction was found. In the present study the ICT wheal reactions had a significantly higher reproducibility than SPT (SD% 17 and 29, respectively). No significant difference between histamine 10 mg/ml (SPT) and 0.1 mg/ml (ICT) was found, but the use of histamine 1 mg/ml in SPT gave significantly less reproducibility than the former two doses (Fig. 2). No advantages of the use of flare compared to wheal in terms of reproducibility were found in the presented data (16).

The slope of the regression line is important as it represents the skin reaction increase as a function of increasing the allergen concentration. The steepest slope was observed in SPT, indicating approximately a doubling of the wheal area for each 10-fold allergen concentration increase. The slope of the ICT wheal and flare was less than that of SPT, with no difference between the former two parameters. Turkeltaub et al. (16) reported an ICT flare slope 5 times steeper compared with wheal reaction, using the sum of diameters. The present study does not confirm these findings. The high value

of the SPT slope might partially compensate for the lesser reproducibility due to a more convincing dose-response curve, indicating that the difference between the skin reaction of the lowest and highest allergen concentration is much larger in SPT than ICT – a valuable point in quantitative skin testing.

The coefficient of correlation of the dose-response reaction in the present study is very high. Except with the use of ICT, flare values exceeding 0.9 were found constantly, indicating that by using the mean of quadruplicate determinations, a highly reproducible skin test can be obtained.

No standardized way of expressing skin sensitivity has been accepted. We have investigated the relationship between end-point and histamine equivalent reaction using SPT wheal as well as ICT wheal and flare. Using SPT, a significant correlation was found, in contrast to ICT. The interpretation of this must be that the two parameters partly estimate two different phenomena, which may be related to the difference in dose-response. The relation between histamine equivalent reaction in SPT and ICT was significantly correlated, indicating estimation of the same phenomena. The correlation, however, reveals the difference in the dose-response curves of the two ways of skin testing, as the flat slope is an expression of a non-constant relationship at different degrees of sensitivity estimated by SPT and ICT.

Contrary to Belin et al. (4) no significant correlation between SPT and ICT end-point titration was found. This might be a consequence of not using the area or not performing linear regression on the titration (4). Another study revealed some degree of correlation between SPT and ICT (6), but the data are not comparable due to difference in the definition of skin sensitivity.

SPT is said to require a 1,000–10,000 more concentrated extract than ICT to produce the same reaction (4, 11). This is in accordance with our own observations (Fig. 6). Using end-point titration, the median difference between SPT and ICT is approximately a factor 500 (range 20–4,000). The same difference was

found using histamine equivalent reaction, but the range is narrower (120–1,200).

Even though the only major difference between SPT and ICT is the amount of allergen/histamine introduced in the skin, some important differences have been revealed. The reproducibility of ICT is superior to SPT, a phenomenon which is only partly a consequence of the larger ICT skin reactions. The slope of the ICT allergen-induced wheal area dose-response curve is significantly flatter than that of SPT. No significant correlation between ICT and SPT end-point titration was found contrary to histamine equivalent reaction. The advantages of SPT in terms of safety, simplicity, minimal discomfort and speed, combined with the steeper dose-response curve, outweigh the advantages of the higher reproducibility of ICT (4, 6, 10). Due to the significantly lower reproducibility of small skin reactions we recommend the histamine equivalent sensitivity as superior to end-point titration. To get optimal information from the skin test we prefer a quantitative skin test titration using quadruplicate determinations of 3–4 10-fold allergen dilutions. An unequivocal dose-response curve (coefficient of correlation > 0.9) and a high reproducibility (SD% < 40%) are mandatory. Provided these criteria are fulfilled and potent allergen extracts are used, we see no advantages in ICT over SPT.

This study was carried out with two different mould species and no difference between them was revealed. However, it cannot be ruled out that the presented data are valid for moulds only, but probably the results are of general validity.

ACKNOWLEDGEMENTS

Extracts of *Cladosporium* and *Alternaria* (Pharmalgen®), histamine and diluents were kindly supplied by Pharmacia, Uppsala, Sweden. The study was supported by grants from Fortsættelsessygekassen Danmarks Sundhedsfond.

REFERENCES

1. Aas, K. & Belin, L.: Standardization of diagnostic work in allergy. *Acta Allergol.* 27, 439–468, 1972.
2. Aas, K., Backman, A., Belin, L. & Weeke, B.: Standardization of allergen extracts with appropriate methods. The combined use of skin prick testing and radio-allergo-sorbent tests. *Allergy* 33, 130–137, 1978.
3. Aas, K.: Some variables in skin prick testing. *Allergy* 35, 250–252, 1980.
4. Belin, L. G. A. & Norman, P. S.: Diagnostic tests in the skin and serum of workers sensitized to *Bacillus subtilis* enzymes. *Clin. Allergy* 7, 55–68, 1977.
5. Brown, H. M., Su, S. & Thantrey, N.: Prick testing for allergens standardized by using a precision needle. *Clin. Allergy* 11, 95–98, 1981.
6. Imber, W. E.: Allergic skin testing: A clinical investigation. *J. Allergy Clin. Immunol.* 60, 47–55, 1977.
7. Malling, H.-J., Andersen, C. E., Boas, M.-B., Holgersen, F., Munch, E. P. & Weeke, B.: The allergy pricker. Quantitative aspects of skin prick testing using a precision needle. *Allergy* 37, 563–567, 1982.
8. Menardo, J. L., Bousquet, J. & Michel, F. B.: Comparison of three prick tests methods with the intradermal test and with the RAST in the diagnosis of mite allergy. *Ann. Allergy* 48, 235–239, 1982.
9. Menardo, J. L., Bousquet, J., Bataille, A., Restagny, G. & Michel, F.-B.: Effects of diluents on skin tests. *Ann. Allergy* 51, 535–538, 1983.
10. Nelson, H.: Diagnostic procedures in allergy. I. Allergy skin testing. *Ann. Allergy* 51, 411–417, 1983.
11. Nierop, G., Voorhorst, R. & Temmerman-van de Vijver, R. L.: Atopic skin test re-evaluated. X. Comparison of a perfected technique of intracutaneous skin testing and the prick test performed with a modified Morrow Brown needle. *Ann. Allergy* 46, 105–109, 1981.
12. Norman, P. S.: *In vitro* methods of study of allergy. Skin and mucosal tests, techniques, and interpretation. In: Middleton, E., Reed, C. E. & Ellis, E. F. (eds.): *Allergy. Principles and practice*, pp. 295–302. C. V. Mosby, St. Louis, Toronto, 1983.
13. Ownby, D. R. & Anderson, J. A.: An improved prick skin-test procedure for young children. *J. Allergy Clin. Immunol.* 69, 533–535, 1982.
14. Ownby, D. R.: Computerized measurement of allergen-induced skin reactions. *J. Allergy Clin. Immunol.* 69, 536–538, 1982.
15. Sussman, G. L., Harvey, R. P. & Schocket, A. L.: Evaluation of skin test response using two techniques of measurement. *Ann. Allergy* 48, 75–77, 1982.
16. Turkeltaub, P. C., Rastogi, S. C., Baer, H., Anderson, M. C. & Norman, P. S.: A standardized quantitative skin-test assay of allergen potency and stability: studies of the allergen dose-response curve and effect of wheal, erythema, and patient selection on assay results. *J. Allergy Clin. Immunol.* 70, 343–352, 1982.

17. Voorhorst, R. & van Krieken, H.: Atopic skin test re-evaluated. II. Variability in results of skin testing done in octuplicate. *Ann. Allergy* 31, 195–204, 1973.
18. Voorhorst, R.: Perfection of skin testing technique. *Allergy* 35, 247–250, 1980.
19. Østerballe, O. & Weeke, B.: A new lancet for skin prick testing. *Allergy* 34, 209–212, 1979.
20. Nordic Society of Allergology: Standardization of allergen extracts. *Acta Allergol.* 28, 219–220, 1973.

Address:

Hans-Jørgen Malling, M.D.
Medical Dept. TA 7511
Rigshospital
Tagensvej 20
DK-2200 Copenhagen N
Denmark

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.