

Perfection of Skin Testing Technique

A Review

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The basic philosophy underlying the daily performance of the atopic skin test on a large scale all over the world, is that we wish to study the reactions of the skin mast cells sensitized with IgE as a prototype of the entire mast-cell system of the body. This atopic skin test, seemingly a very simple method, nevertheless requires a very sophisticated set-up and, like most laboratory techniques, many precautions.

The size of the skin reactions, the ultimate result of the interaction between the allergen and the specifically sensitized mast cell, is determined by the amount of allergen injected, the degree of sensitization of the mast cells, and the reactivity of the skin to mediators (especially histamine (12)) liberated as a consequence of this interaction. In the following, a number of aspects of the testing procedure will be discussed briefly.

USE OF SYRINGES AND INJECTION NEEDLES

In agreement with the report of the Committee on Standardization from the American College of Allergists (5), we found that the Hamilton 0.1 ml syringe is the most accurate (for details see: 12). The amount of fluid that is injected into the skin varies from 0.005 to 0.05 ml. All the relevant factors taken into account, 0.02 ml is considered to be the optimal dose.

We also investigated a number of different sizes and types of bevel. The 30-gauge needle (Becton and Dickinson) with a metal hub, Luer lock 3/8" with a flat front and two secondary lateral side bevels, proved to be the most satisfactory.

INJECTION SITES

The reactivity of the skin to an allergenic extract is not the same all over the body (Table 1). This divergence was ascribed by Swain & Becker (9) to differences in skin sensitivity to histamine. There is also a circadian rhythm in the size of the skin reactions to allergens and histamine, which are maximal during the night (8), and show, at least for skin reactions to histamine, a variation corresponding to the menstrual cycle in women in their fertile years (3).

Sites of skin tests are often taken too close together. Under these conditions, the stronger skin reactions influence the weaker ones, probably due to some kind

Table 1
Skin reactivity to allergens

(according to Alexander & MacConnell (2), Bowman (4), Swain & Becker (9))	
Forearms	volar parts > dorsal parts ulnar parts > radial parts volar parts < back and abdomen most reactive: cubital fossa least reactive: wrist
Legs	lateral parts thigh < back and abdomen most reactive: popliteal fossa

of axon reflex (12). To prevent this side effect, the sites should be at least 5 cm apart.

Studies performed in octuplicate (13) showed that skin tests can be done best on the skin of the back, and although the skin reactions on the different fields of the back do not have the same shape, the parameter (length + width) of the reactions did not vary significantly between fields.

COURSE OF THE DEVELOPMENT OF THE SKIN REACTIONS (Fig. 1)

Morphologically, the atopic skin reaction does not differ from the skin reaction to histamine except that the latter reaches a maximum 8-10 min after the injection, whereas the maximum reaction to allergens is seen after 12-15 or 17 min after the injection and, probably

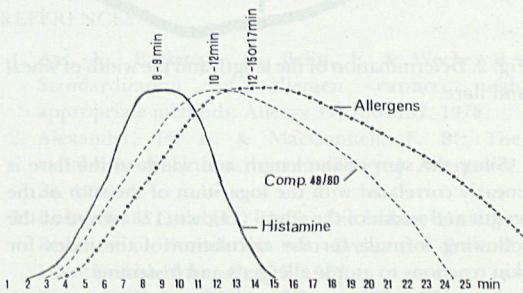


Fig. 1. Course of the development of the skin reactions to histamine, compound 48/80, and atopic allergens.

because of a more gradual liberation of histamine, persists at a constant level much longer. The maximum reaction to compound 48/80 occurs between 10 and 12 min after the injection. The divergence this gives in the shape of the time-response curve is important, and forms one of the major obstacles to the establishment of correlation between the strength of the allergen-reagin reaction and that of histamine.

MEASUREMENT OF THE SKIN REACTIONS

To obtain skin reactions of equal size, allergen extracts used in the intracutaneous technique should be 1000 times weaker than the dose used in the prick test (prick-through-drop method) (6). It is nonsense to claim that intracutaneous tests are more dangerous; the factor of 1000 should only be taken into account. We prefer the intracutaneous method for many practical purposes, and it offers the added advantage that the biological scatter is smaller (13).

To obtain the most reliable parameter for the size of the skin reactions, the outline of both the wheal and the flare is drawn on the skin with a ball-point pen and then the ink marks are traced on to a piece of transparent tape which is attached to paper for measurement (Fig. 2).

We obtained the best parameter by using the sum of length and width of the wheals and the flares.

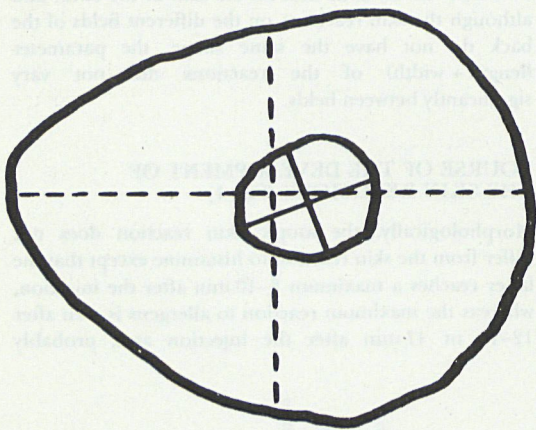


Fig. 2. Determination of the length and the width of wheal and flare.

Since the sum of the length and width of the flare is linearly correlated with the logarithm of the sum of the length and width of the wheal (14), we (17) arrived at the following formula for the calculation of the index for skin reactions to atopic allergens and histamine:

Index = $\frac{1}{2}\{(L + W)_{\text{flare}} + 3.7 + 11.5 \log (L + W)_{\text{wheal}}\}$
for compound 48/80:

Index = $\frac{1}{2}\{(L + W)_{\text{flare}} + 5.8 + 7.5 \log (L + W)_{\text{wheal}}\}$

The use of these indices, which are actually the result of four parameters, proved to be very valuable for any quantitative work with skin tests. Because, notwithstanding all precautions, there is a certain degree of scatter, we recommend performance of the test with replicates, preferably in a series of three 10-fold dilutions.

For practical purposes, the technique just described is too complicated. We have demonstrated (15) that in routine praxis, subjective scoring is acceptable (correlation with the objective method: $r = 0.98$). At any rate, we recommend the use of three 10-fold dilutions, the best parameter being the sum of the three scores.

USE OF COMPOUND 48/80 AS REFERENCE TEST

Scandinavian authors (1) think it preferable to "correct" the measurements of the atopic skin test with the result of a test with histamine. We do not think so, for the following reasons:

First of all, when the atopic skin reaction is at its maximum (at approximately 15 min) the reaction to histamine has already passed its prime (maximal at 8 min). When histamine is included as reference test, two readings must be performed, one after 8 and the other after 15 min. To get round this difficulty, we have recommended the use of compound 48/80, which can be read simultaneously with the atopic skin reactions. The correlation between the size of skin reactions to histamine and compound 48/80 is very high: $r = 0.83$ (between histamine duplicates: $r = 0.91$ and between compound 48/80 duplicates: $r = 0.87$) (18).

Furthermore, it is questionable whether atopic patients (apart from those with, for example, severe atopic dermatitis) vary considerably in the skin response to histamine (18). This raises the question of whether the just-mentioned correction for the skin reactions is even necessary. We should not forget that by applying it we introduce another biologic variable. Moreover, a study in four groups of atopic patients has taught us that the correlation between the test results to allergens (grass pollen, *Dermatophagoides pteronyssinus*) and to compound 48/80 is not very high. The correlation coefficients (r) varied from 0.33 to 0.40 and the correlation should be qualified, according to the statisticians, as "very low" (0–0.45)! We think that the importance of the use of compound 48/80 as reference test lies in the fact that because the strength of this test solution does not vary, we have, especially when the reactions are scored subjectively, a good check on the degree to which the "inner standard" used for subjective scoring remains fairly constant or shifts in any particular direction in the course of time (16).

BIOASSAY OF THE STRENGTH OF TEST EXTRACTS

Perfection of the skin testing technique is especially

valuable for the bioassay of allergen extracts of unknown strength against standard extracts. The difference in strength can be calculated on the basis of the distance between the log-dose response lines. When the just-described perfected technique of skin testing is used, only a small group of subjects (say, five) is necessary for a sufficiently reliable bioassay (11). It should be emphasized, however, that the log-dose response curve only approaches a straight line when the "indices of skin reactions", calculated as described here, do not exceed the limiting values of 6 and 12.

HOW TO PREVENT A GRADUAL DECREASE IN STRENGTH

It is self-evident that a number of measures are required to prevent a decrease in the strength of the extracts in the course of time. We recommend weekly preparation of the diluted test extracts from more concentrated stock extracts and refrigeration when not in use. Unused stock extracts can be kept for a fairly long time in the deep-frozen state. Generally speaking, the most concentrated and crude test extracts are the most stable, and from this point of view purification is not always an advantage.

Another problem, one which is not always realized, is that when allergenic test extracts are calibrated against new standard extracts over and over again, the standards will gradually become weaker and weaker. To prevent this, continuous monitoring of the test results obtained in a large population of atopic patients is required. As we have reported elsewhere (16), this is possible within certain limits. As shown in the same paper it also is possible, on the basis of a statistical analysis of the test results of a large population of atopic patients, to make test extracts containing different allergens more or less biologically equivalent.

INTERPRETATION OF THE TEST RESULTS

Interpretation of test results can only be considered of value when skin testing is done with all the necessary precautions and with extracts kept at equal strength as much as possible. But even then it must be kept in mind that if the extracts are chosen too strong, we will find too many cases with positive reactions without clinical symptoms after contact with the relevant allergens, and patients who are extremely atopic to the allergen in question will run the risk of general reactions. When the chosen extracts are too weak, too few patients with allergic complaints will be found after contact with the allergen. It is the clinician's task to fix the strength of the allergen-test extracts such that the predictive value is as high as possible. When this is achieved, the accuracy of predictions will be 60–70% in patients with moderate skin reactions and up to 95% in the patients with stronger reactions (7, 10).

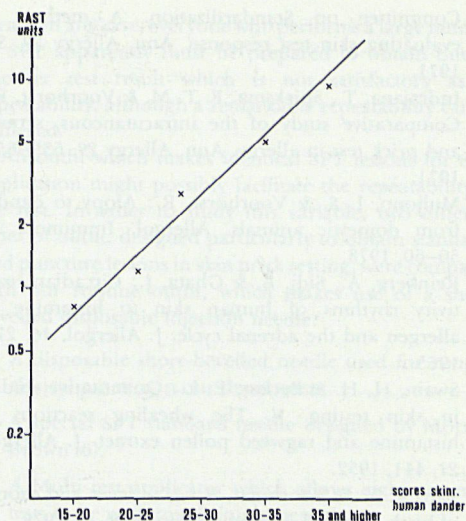


Fig. 3. Linear correlation between skin reactions and the serum IgE level (56 observations). The RAST-tests were performed for us by Dr. R. Aalberse, "Bloedtransfusie Dienst", Amsterdam.

RELATION BETWEEN ATOPIC SKIN TEST AND SERUM IgE level

In larger groups of patients we found (Fig. 3) a linear relationship between the indices of skin reactions and the logarithm of the IgE titre. This means that when the skin test is done with stronger test extracts, the results may be positive in more weakly sensitized patients, although no IgE can be demonstrated with the RAST.

Furthermore, it seems *a priori* that the degree of a patient's clinical sensitivity to a certain allergen is reflected much better by the skin test which demonstrates the reactivity of the mast cell than by the RAST. It is not a *conditio sine qua non* that the sensitization of the mast cells be linearly proportional to the serum levels of specific IgE in every case of atopy.

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Some Variables in Skin Prick Testing

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Skin prick testing (SPT) represents an indispensable tool for the routine diagnostic work in allergy. It is the skin test method which has the highest degree of diagnostic specificity provided satisfactory allergen preparations are used (2,4,7,10). SPT is considered also to be the most lenient of the different skin test techniques. Only minimal amounts of the actual allergen are introduced into the skin during SPT, and the technique implies very small risks for serious reactions (3, 5, 7, 8).

For SPT to be applicable as bio-assay in scientific work and in standardized procedures, the variables of the method must be known and controlled. For this purpose, we have performed several studies, many of

them unpublished, in addition to borrowing data from others.

Histamine appears to be the mediator which determines the local allergic reaction to skin testing, and is well suited as reference (3,9,12). Histamine hydrochloride 1 mg/ml (histamine 5.43 mmol/l) has been established as the standard reference for skin prick testing by the Northern (Scandinavian) Society for Allergology (1,12).

There is some diversity in the patient population as regards the reactivity to histamine in addition to the wide distribution of immunological reactivity to various allergens. The use of a given histamine reference com-

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