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PATCH TEST METHODS

I. A comparative study of six different types of patch tests

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Much time can be saved by using standardized test units on large-scale epicutaneous testing of patients. Several types of patch test plasters are commercially available. These vary considerably in regard to size and quality. The purpose of this study has been to investigate the influence on the test response of the variations in shape and material of two test plasters and four other patch test units by means of both allergic and toxic test reactions. In addition the reproducibility of results obtainable with these patch tests was estimated.

Material and methods

Patients.—Sixty patients were tested, thirty with a toxic substance, while allergic reactions were provoked in the others. Only patients who at the time of testing had apparently normal skin on the back were used in the study.

Test solutions.—Benzalkonium chloride of a 1.5 per cent concentration in water was found to provoke toxic skin reactions of suitable intensity. The allergic responses were provoked with the specific allergens to which the patients had reacted earlier. These included mainly potassium bichromate, nickel sulfate and paraphenylenediamine. The allergen concentrations were adjusted to produce, if possible, relatively slight reactions.

Patch test units.—Of the six different types of test patches two were commercial test plasters and the others were different patch test units employed in Sweden. The various types of patches and relevant data are seen in figure 1 and table 1.

Test method.—The patch tests were placed on the upper part of the back in two vertical rows 5 cm from the midline, each row including three paches. To avoid systematic error due to the placement of the individual pieces, they were applied to each group of 15 patients according to a pattern shown in table 2.

One drop of test solution was applied to each patch. In order to obtain as accurate an amount as possible pipette bottles, delivering standardized quantities (2) were employed. The test patches were left in place for 24 hours. The readings for the benzalkonium tests were made one hour after the removal of the patches and those for allergic reactions after 24 hours.

The intensity of the skin reactions was rated from 0 to 3 (erythema = 1; erythema + infiltration = 2; erythema + papules and/or vesicles = 3).

Statistical methods.—The results obtained with the six patch test units, A, B, C, D, E and F were compared in pairs, each one against the others at the three different levels. This required a group of 15 individuals, if three comparisons, one at each level, were to be made for each individual. The experimental data included 2×15 individuals in each of the groups "toxic reactions" and "allergic reactions".

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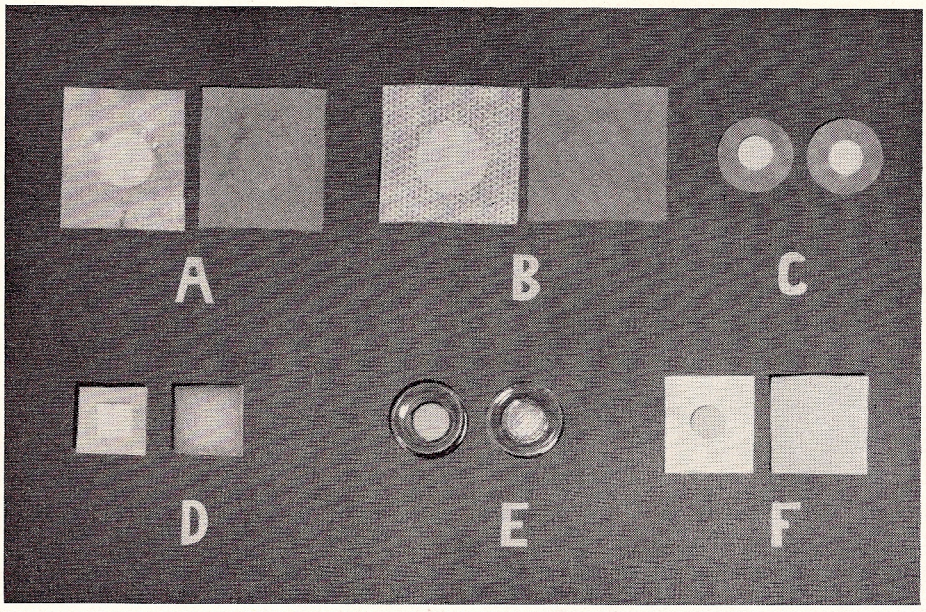

Fig. 1. Patch tests investigated.
A = Leucotest®; B = Porotest®; C = Cellulose-test; D = Pressure-test; E = Glass-test; F = "Aluminium" test.

Table 1. *Patch tests investigated*

Name	Source	Insulation	Absorbent material		
			Type	Area (mm^2)	Absorption capacity (ml H_2O)
A. Leucotest®	P. Beiersdorf & Co., Hamburg	Cellophane	Cotton	75	0.06
B. Porotest®	Lohmann, K. G. Fahr/Rhein	None	Cellulose	330	0.05
C. Cellulose-test	S.-G. Blohm, Stockholm	Cellulose	Cellulose	79	0.04
D. Pressure-test	Å. Fernström, Stockholm	Rubber cloth	Cellulose	79	0.04
E. Glass-test	S.-G. Blohm, Stockholm	Glass	Sintered glass	79	0.04
F. "Aluminium"-test	S. Fregert, Lund	Polyethene-coated aluminium	Cellulose	110	0.04

The comparison of each pair of reactions at a certain level was designated as shown in table 1.

Weaker reaction on the left side than on the right = 0; the same reaction on both sides = 1; stronger reaction on the left side than on the right = 2.

A difference in reaction intensity between the sides of at least one unit was inter-

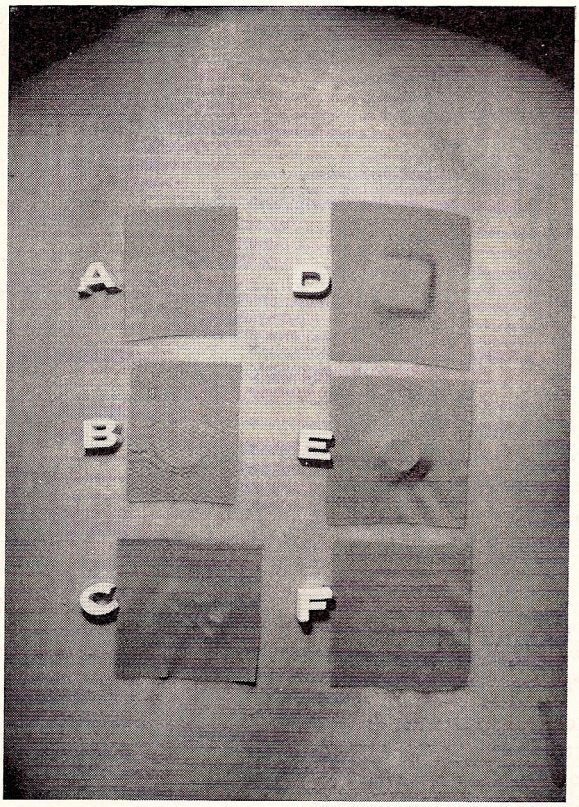

Fig. 2. Example of position of the patch tests on the back of a patient.

preted as a difference in the paired comparisons. The results obtained with such a paired analysis of one experimental group of 15 persons are presented in table 3.

Statistical analysis

I. *Toxic reactions.*—With the χ^2-test no significant difference between the three levels or between the two groups of 15 patients was found. The numerical values could consequently be added and the results found in table 4 were obtained. Each figure represents the results of 30 comparisons.

Assuming that "no difference between the groups" exists the expected value for each comparison = 1, and the expected value for each sum (S) would be: E (S) = 30. Any deviation from this value can be tested, since each sum has approximately a normal distribution. The variance of the distribution can be estimated according to the following:

At *one* comparison the probability distribution is:

value x: 0 1 2

probability : p 1−2 p p;

and hence $\mu_x = 1$ and $\sigma^2 x = 2$ p.

For a sum S of independent comparisons

$\mu_s = n$ and $\sigma_s^2 = 2$ np.

An observed value can be tested with regard to its deviation from the mean by $u = \frac{S - n}{\sqrt{2\ np}}$ where p has been estimated at 1/4.

The *results* of the analyses showed that: A was significantly "stronger" than expected ($p < 0.01$) and B and C significantly "weaker" than expected ($p < 0.01$ resp. $p < 0.05$).

Table 2. *Patterns employed for application of test patches per group of 15 persons*

1	2	3	4	5
A B	E F	C D	A C	D F
C D	A B	E F	B E	A C
E F	C D	A B	D F	B E
6	**7**	**8**	**9**	**10**
B E	A D	C E	B F	A E
D F	B F	A D	C E	B D
A C	C E	B F	A D	C F
11	**12**	**13**	**14**	**15**
C F	B D	A F	D E	B C
A E	C F	B C	A F	D E
B D	A E	D E	B C	A F

Table 3. *Results of paired comparisons of the skin reactions in a group of 15 patients who were investigated for toxic response with the six types of patch test units*

A–F = Designation of patch test unit
0 = Weaker reaction on left side than right
1 = Same reaction on the two sides
2 = Stronger reaction on left side than right

		Right side						
		A	B	C	D	E	F	Sum
Left side	A	–	2	2	1	2	1	8
	B	0	–	1	0	0	1	2
	C	0	1	–	1	0	1	3
	D	1	2	1	–	1	1	6
	E	0	2	2	1	–	0	5
	F	1	1	1	1	2	–	6

No significant difference between the other patch test units was noted.

II. *Allergic reactions.*—The results of the allergen testing were analyzed in a manner analogous to that described for the toxic reactions. Each comparison sum, as in the other analysis, represents 30 paired comparisons; they are presented in table 4.

For one sum S, $0 \leq S \leq 60$;

the mean value $E(S) = 30$ and the variance is $\sigma_s^2 = 2\,np$, where $n = 30$ and $p = 1/4$. The zero hypothesis, i. e. that all the allergens had the same effect, can be examined as follows:

$u = \frac{S - E(S)}{\sqrt{2\,np}}$; u is approximatively normally distributed N (0.1). The critical value for the sum is 30 ± 7.8.

The *results* of these analyses showed that B and C were significantly "weaker" than expected ($p < 0.05$) and A, D, E and F could not be differentiated statistically.

Results

The statistical analysis and table 4 show that when eliciting toxic responses the patch test unit A (Leucotest®) gave significantly stronger reactions and that type B (Porotest®) gave significantly weaker reactions than expected.

When eliciting allergic responses, patch test units B (Porotest®) and C (Cellulosetest) gave significantly weaker reactions than expected.

With the other types of patch test units no significant differences were noted in either of the responses—toxic or allergic.

Reproducibility of test responses

A separate estimation of the error of the method was carried out by analyzing the toxic responses to the six types of test patches. Each was applied symmetrically in duplicate on the back of 35 patients not included in the comparative study. The skin reactions were provoked with benzalkonium chloride at 1.5 % and their classification and readings were performed as described in the methods.

Error of the method.—For the paired tests the method error was calculated from $\sqrt{\Sigma d_i^2 / 2n}$, where d_i represents the individual differences. The values obtained may be found in table 5. In the table the relative error of the method as the per cent of the total median value (about = 2) of the whole material is also given.

Table 4. *Statistical analysis of the paired comparison sums obtained by testing with six different patch tests on 60 patients*

Patch test unit	Sum of paired comparisons	
Designation Type	Toxic reactions	Allergic reactions
A. Leucotest®	48	36
B. Porotest®	8	18
C. Cellulose-test	21	22
D. Pressure-test	34	35
E. Glass-test	32	32
F. "Aluminium"-test	35	37

Table 5. *Error of the method of six different patch tests estimated by simultaneous duplicate applications of benzalkonium chloride at toxic concentration (1.5 %) on 35 patients*

Patch test	Method Error	
	Abs.	%
A. Leucotest®	0.34	17.0
B. Porotest®	0.24	12.0
C. Cellulose-test	0.19	9.5
D. Pressure-test	0.18	9.0
E. Glass-test	0.29	14.0
F. "Aluminium"-test	0.19	9.5

The errors of the method for the six types of patches were compared by means of the F-test. The test for significance at the 5 per cent level showed that the error of the method for the Leucotest® was significantly greater than that for all the others except for the glass-test. The error of the method for the glass-test was significantly larger than for the cellulose-test, pressure-test and "aluminium"-test. All of the other differences were not significant.

Discussion

Joseph Jadassohn appears to have been the first to use patch testing for the demonstration of epicutaneous allergy (6). This was in 1895 and sixteen years later Bruno Bloch described the method (1). The principle of the test method according to Jadassohn–Bloch is simple. A piece of linen about 10 × 10 mm is moistened with the substance to be investigated at a subtoxic concentration. The piece is placed on the skin and covered with an overlapping impermeable membrane and fixed to the skin with tape for 24–48 hours.

Various modifications of this method have been described, i. a. those of Rokstad (8), Fernström (5) and Blohm (3). In most investigations the original method of Jadassohn–Bloch has been used.

One of the difficulties encountered in judging the results of reports where the authors only state that they employed the Jadassohn–Bloch technic is that available handbooks describe the details of the method differently. For example Mayer (7) in Jadassohn's handbook states that the test solution should be applied to a piece of linen 1.3 × 1.3 cm (169 mm^2). In the recently published supplement to this handbook Burckhardt (4) recommends "a small piece, e. g. 0.5 × 0.5 cm (25 mm^2)", whereas Spier (9) in Gottron and Schönfeldt's handbook proposes 1.0 × 1.0 cm (100 mm^2). Accordingly the test patches vary in size up to six times. One drop of the test solution, which is most commonly recommended, can consequently provide very different amounts of allergen per mm^2 of skin depending on the method followed.

The varying results obtained in the present study with Leucotest® (A) and Porotest® (B) patches can in part be explained by the mentioned discrepancies. In the first instance the skin area exposed to the allergen was approximately 75 mm^2 and in the latter about 330 mm^2—a fourfold difference.

Different structures of the test patches in other respects can also contribute to skin responses of differing intensity. The two types of commercial test plasters mentioned not only differ greatly in the size of the absorbent part but also in regard to its insultation from the adhesive tape. In the Porotest® there is no isolation between the absorbent material and the adhesive tape. Leakage of the test substance through the test plaster can occur. This may give false negative test responses. We have noted this in testing formalin-sensitive patients.

In table 4 it can be seen that the comparison sums for the toxic reactions have a larger spread than those for the allergic responses. The reason for this finding is unclear. The low values for patch test C (Cellulose-test unit) may be explained by the thinness of the absorbent material in the units used in the present study. Subsequently the absorbent material has been made thicker to get this patch test unit more comparable to D, E and F.

The obtained comparison sums do not indicate per se which of the test patches is "best". They can however, when considered in relation to the method errors, offer information of value for the interpretation of results where one or another of these patch test units have been employed.

SUMMARY

A comparative statistical analysis has been carried out on the toxic and allergic skin responses obtained when testing with six different types of patch tests. Thirty patients were tested with an allergen to which they were sensitive and an equal number of persons were tested with benzalkonium chloride in a toxic concentration.

Significant differences in the intensity of the reactions were noted, and these could be related to construction details of the patch test units, such as size and thickness of the absorbent material and the method of insulating this from the fixing tape.

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