Acta derm.-venereol. 46: 153-158, 1966

ROUTINE PATCH TESTING II*

Proposed basic series of test substances for Scandinavian countries and general remarks on testing technique

BERTIL MAGNUSSON, SVEN-GÖSTA BLOHM, SIGFRID FREGERT, NIELS HJORTH, GUNNAR HØVDING, VEIKKO PIRILÄ AND ERIK SKOG

At the meeting of the Northern Dermatological Society held in Gothenburg in 1962 one of the main themes was methods for epicutaneous testing. These vary considerably in the Scandinavian countries, and it was obvious that greater uniformity was desirable. Such uniformity would permit a more realistic future collection of data and comparison of test results in the Scandinavian countries and would facilitate the recognition of national differences and fluctuations in the "allergenic environment". For this purpose a committee was elected at the meeting in 1962 and was given the responsibility of formulating a proposed scheme for standardization of routine patch testing (12).

There exists a number of modifications of the original Jadassohn-Bloch method, but the committee for the time being recommends the traditional method for routine use. The principle is simple, but there is no clear description with precise presentation and exact indication of the desirable size and composition of the test patches, etc. in the literature. Information is also lacking on such details as the most suitable test region, the most effective exposure time or the time for final readings.

The suggestions and recommendations for performance of patch testing given in the present report take into account the information in the literature as well as some of our own investigative results. In addition we will discuss ten test substances which are known to be common contact allergens in Scandinavia. These test substances we recommend for the present as a minimum series for initial screening of patients with contact dermatitis.

I. Patch test material

1. *Carrier material*

Suitable for routine test patches are pieces of standardized filter paper, for example, Whatman filter paper No. 3 MM.

A handy diameter for the patch is 10 mm, this being the median value calculated from the test patches which are in use at a number of dermatological clinics. A comparison of the intensity of skin reactions obtained with a drop of about 35 microliters using different sizes of patches indicates that a patch of 10 mm in diameter induces the strongest reactions (11). In Finland 7×7 mm patches, with about 15 microliter solution per piece, are employed (18).

* *Report* 2 from the Scandinavian committee for standardization of routine patch testing (12). Address of the chairman of the committee: Bertil Magnusson, M. D., Department of Dermatology, Sahlgrenska Sjukhuset, Gothenburg SV, Sweden.

2. *Insulation material*

The material protecting the patch from the fixation plaster must extend beyond the patch edge a distance related to the size of the patch itself, minimally about 5 mm. Because of the relatively frequent sensitivity to the traditional rubberized material another type of insulation should be employed, for example, polyethylene-coated aluminium foil, cellulose or possibly cellulose acetate foil.

3. *Adhesive material*

It is difficult to find a tape which is suitable for fixation of the test patches. If the plaster is not sufficiently impervious to air and moisture and does not adhere adequately to the skin there is a danger of false negative test results (11). On the other hand the better the plaster sticks to the skin the greater is the risk of skin irritation resulting in nonspecific test responses (13). No ideal plaster material for this purpose is at present available but research in this field has been intensive during recent years particularly in the USA.

II. Patch test procedures

1. *Dosage*

The dosage of the test substances must be as exact as possible and in a quantity which suits the surface, size and composition of the test patches. For precision application of solutions a dose pipette flask has been elaborated by Blohm (4) and modified by Pirilä (17). Special syringes for dosing of test solutions have also been described by Hjorth and Trolle-Lassen (10). For quantitative routine tests with ointments a method using aluminium tubes, with special "non-soiling stoppers", has been designed by Blohm (4).

2. *Test site*

The back, the front of the thigh, and the arms are the skin regions most often employed for patch testing. Since test responses can vary in intensity in different skin regions, it is most important in comparative investigations to confine the testing to one test region if possible. Skin responses on the back are significantly more intensive than reactions on the arms or thighs (15).

3. *Exposure time and reading of skin responses*

The test patches should remain in place for 24 or 48 hours. Preliminary readings may be made about an hour after removal of the patch, but it is most desirable to make readings one day and, if possible, also 3 to 4 days after the removal.

III. Scandinavian basic series of patch test substances 1964

1. *Selection of test substances*

Instead of a large battery of test substances we have selected a small series made up of ten agents known to be common causes of allergic contact dermatitis in the Scandinavian countries (table I). This *"Basic P. T. Series, 1964"* is included in the larger routine series presently in use at the various departments of dermatology in Scandinavia. Table I presents the recommended concentrations and vehicles as well as sources of these substances.

A basic p.t. series of the described type is usually adequate for preliminary routine screening of patients with contact dermatitis of suspect allergic genesis. For a more penetrating examination one should test the patient with additional substances. In order to facilitate the choice of substances for such extended screening tests the committee will subsequently suggest a supplementary series of other substances also found quite frequently to be causes of contact dermatitis in the Scandinavian countries.

In the investigation of a patient with occupational contact dermatitis the testing should from the beginning aim at substances which occur in his place of work. Concomitantly a standard test series should be employed.

Table 1. *Scandinavian Basic Patch Test Series, 1964*

No.	Test substance	Description	Source	Vehicle	Weight %	Molarity[6] (approx).
1.	Potassium bichromate	$K_2Cr_2O_7$	Hopkin & Williams, 6971 Essex, England	H_2O[1]	0.5	0.017
2.	Cobalt chloride	$CoCl_2 . 6H_2O$	BDH, Analar Poole, England	,,	2.0	0.086
3.	Nickel sulfate	$NiSO_4 \cdot nH_2O$	Hopkin & Williams Analar (Ni: 20.7–21.9 %)[5] Essex, England	,,	5.0	0.19
4.	Formaldehyde	CH_2O	Baker & Adamson, 1778 New York, N. Y., USA	,,	2.0	0.68
5.	p-Phenylenediamine (PPDA)	$C_6H_8N_2$	BDH, Laboratory reagent Poole, England	Petrolatum[2]	2.0	0.19
6.	Mercaptobenzothiazole (MBT)	$C_7H_5NS_2$	Merck, Darmstadt 5996 West-Germany	,,	2.0	0.12
7.	Tetramethylthiuram-disulfide (TMTD)	$C_6H_{12}N_2S_4$	Fluka, purum Buchs, Switzerland	,,	2.0	0.085
8.	Balsam of Peru	Pharm. Nord. 1964	—	,,	25.0	—
9.	Turpentine	Mixture, oxidized[3]	Dept. of Allergology Karolinska Hosp., Stockholm, Sweden	Peanut oil[4]	10.0	—
10.	Neomycin sulfate	Lot 110	H. Lundbeck & Co., Copenhagen, Denmark	Petrolatum[2]	40.0	—

[1] Distilled in glass-lined apparatus.
[2] Vaselinum flavum. Pharm. Nord. 1964.
[3] Oxidation controlled by iodine titration + n/25/D. C:a 1 mol. % perox. (3).
[4] Oleum arachidis. Pharm. Nord. 1964.
[5] $=NiSO_4$ c:a 56 %.
[6] Calculated from the formula.

The allergens which may be encountered in different occupations and other contact allergens with recommended concentrations and vehicles are listed i. a. in the following reports: Bonnevie (5); Schwartz *et al.* (22); Blohm *et al.* (2); Burckhardt (6); Pirilä and Salo (19).

2. *Storage*

The test substances should be stored in sealed vessels and shielded from direct sunlight. These safeguards are essential particularly for organic substances.

3. *Stability*

For the majority of the test substances the stability has not been examined closely, but it is advisable to renew the solutions monthly and the ointments every third month (2). The intervals may be lengthened if tests are only made occasionally and containers with adequately tight seals are employed.

Comment

In large scale testing of patients much time is saved if ready-made test units are employed. Attempts to improve and standardize the test units in regard to design and material are attempted in many places. One modification which has been elaborated to increase the contact between the allergen and the skin is the so-called pressure patch test (7). Rubber cambric has been used as insulation material in this

unit, but trials are presently being made to replace this material with another which is more inert than rubber. Two models employing nonallergenic materials (glass and cellulose) for this purpose have been proposed by Blohm (1, 4).

There is on the market a large number of so-called "test plasters".* These vary greatly in material and form. Comparative investigation of these test units and plasters showed significant result-differences which could be related to variations in their make-up. Thus Porotest® gave significantly weaker test reactions than the others (14).

For several of the different test substances included in routine patch series and in part also those comprising the recommended series in this report there is only limited information available on the chemical portion which induces the sensitization. The allergenic factors in paraphenylenediamine for example are still unknown. According to Spier (24) the substance should be colored to be effective but conclusive data are still lacking. Probably there is involved an auto-oxidation process—such as with turpentine—which accelerates the course of the reaction (16).

A great deal of work has been devoted to the isolation of the sensitizing fraction in turpentine. We have learned, primarily through the investigations by Hellerström *et al.* (8) and Pirilä *et al.* (20, 21), that the allergenically active factor results from oxidation and that a certain fraction of an oxidized 3-carene is probably the reactive component.

In regard to sensitizing agents among the metal salts, potassium bichromate is available in a well-defined pure form. On the other hand cobalt chloride and nickel sulfate cannot be obtained without reciprocal traces of cobalt or nickel. Furthermore, the amount of water of crystallization varies, whereby the allergen concentration in the test solutions is influenced.

Balsam of Peru comprises, like other substances in the proposed series, a mixture of a large number of chemical components. For details the reader is referred to Hjorth's work (9).

The commercial product neomycin is a mixture of neomycin B and C. It is mentioned that when testing for neomycin, positive skin reactions are sometimes more delayed than when using other substances and the reactions may be relatively weak.

In this report we have purposely chosen only to present a few general recommendations simply because basic research in this field is very limited. Investigations on the technical details of patch testing are, however, in progress, and we hope to offer more concrete suggestions in later reports.

In some countries, e. g. USA and England, the attitude towards battery tests has been hesitant. Testing has instead been limited to one or several substances suggested by the patient's history. The routine in Scandinavia and also in central Europe is to use an extensive patch test series for screening, supplemented by patch tests with the particularly suspect substances (5). The experience of the members of the committee with the extensive screening tests is favorable. Valuable information on the agents inducing the contact dermatitis and possible cross-sensitization may be revealed in patients where the history alone would never lead to its detection.

An objection to the routine use of an extensive patch test series is that it implies unnecessary exposure of patients to a large number of potent contact allergens, possibly with unfortunate consequences. The risk of sensitization with properly performed patch testing can, however, according to experience to date, be considered minimal (23).

We would like to emphasize particularly that the present selection of the test substances for a basic test series does not represent a definitive choice. At present we have endeavored to determine which allergens play the most important aetiological

* Among those available in Scandinavia are Leucotest® (Beiersdorf), Porotest® (Lohmann) and Lysa Special-plaster® (without absorbent carrier) (Nopi).

role in contact dermatitis in Scandinavia – with the understanding that the allergenic environment is constantly changing.

SUMMARY

It is difficult to compare patch test results from different clinics since the methods are not uniform. A Scandinavian committee has therefore been formed to study this problem and make recommendations.

In this paper the committee has presented certain information on techniques for patch testing and suggest the use of a preliminary basic Scandinavian patch test series composed of ten currently common contact allergens. They are: potassium bichromate, cobalt chloride, nickel sulfate, formaldehyde, para-phenylenediamine, mercaptobenzothiazole, tetramethylthiuram-disulfide, balsam of Peru, turpentine and neomycin sulfate.

For wider screening purposes this series is considered incomplete.

REFERENCES

1. Blohm, S.-G.: Storage of epicutaneous test solutions; in *Proc. XII Intern. Congr. Dermat.*, vol. 2, p. 1521, 1963. Excerpta Medica, New York.
2. Blohm, S.-G., Fernström, Å., and Rajka, G.: Tabell över lösningar och ämnen för epikutantestning; with Hellerström, S.: *Hudsjukdomar.* Håkan Ohlsson, Lund, 1964.
3. Blohm, S.-G. and Widmark, G.: Oxidation of Δ^3-Carene. *Acta Chem. Scand. 9:* 920, 1955.
4. Blohm, S.-G.: Personal communication.
5. Bonnevie, P.: *Aetiologie und Pathogenese der Ekzemkrankheiten.* Nyt Nordisk Forlag, Copenhagen 1939.
6. Burckhardt, W.: Die beruflichen Hautkrankheiten; in Jadassohn, J.: *Handbuch der Haut- und Geschlechtskrankheiten,* vol. 2, pt. 1, p. 369. Springer-Verlag, Berlin 1962.
7. Fernström, Å.: Patch-test studies. A new patch-test technique. *Acta derm.-venereol. 34:* 203, 1954.
8. Hellerström, S., Thyresson, N., Blohm, S.-G., and Widmark, G.: On the nature of the eczematogenic component of oxidized Δ^3-carene. *J. invest. Derm. 24:* 217, 1955.
9. Hjorth, N.: Eczematous allergy to balsams, allied perfumes and flavouring agents. With special reference to balsam of Peru. *Acta derm.-venereol.* Suppl. 46, 1961.
10. Hjorth, N. and Trolle-Lassen, C.: Quick and easy method for application of patch tests and storage of test substances; in *Proc. Northern Dermat. Soc. p. 101.* Acta derm.-venereol. Stockholm 1962.
11. Magnusson, B.: Patch test technique; in "Allergic Contact Eczema in Theory and Practice". *Acta derm.-venereol. Extra* suppl. 1966.
12. Magnusson, B., Blohm, S.-G., Fregert, S., Hjorth, N., Høvding, G., Pirilä, V., and Skog, E.: Standardization of routine patch testing. Report 1; in *Proc. Northern Dermat. Soc.* p. 126. Acta derm.-venereol. Stockholm 1962.
13. Magnusson, B. and Hellgren, L.: Skin irritating and adhesive characteristics of some different tapes. *Acta derm.-venereol. 42:* 463, 1962.
14. Magnusson, B. and Hersle, K.: Patch test methods. 1. A comparative study of six different types of patch tests. *Acta derm.-venereol. 45:* 123, 1965.
15. Magnusson, B. and Hersle, K.: Patch test methods. 2. Regional variations of patch test responses. *Acta derm.-venereol. 45:* 257, 1965.
16. Mayer, R. L.: Group-sensitization to compounds of quinone structure and its biochemical basis; role of these substances in cancer. *Progr. in Allergy, Fortschr. der Allergielehre 4:* 79, 1954.
17. Pirilä, V.: Personal communication.
18. Pirilä, V. and Kajanne, H.: On standardization of epicutaneous tests. *Acta Allergol.* 19: 283, 1964.
19. Pirilä, V. and Salo, O. P.: Exposure to some contact allergens; in *Proc. Northern Dermat. Soc.* p. 75. Acta derm.-venereol. Stockholm 1962.
20. Pirilä, V. and Siltanen, E.: On the chemical nature of the eczematogenic agent in oil of turpentine. III. *Dermatologica 117:* 1, 1958.
21. Pirilä, V., Siltanen, E., and Pirilä, L.: On the chemical nature of the eczematogenic agent in oil of turpentine. IV. The primary irritant effect of terpenes. *Dermatologica 128:* 16, 1964.
22. Schwartz, L., Tulipan, L., and Birming-

ham, D.: *Occupational Diseases of the Skin.* 3rd Ed. Lea and Febiger, Philadelphia, 1957.

23. Skog, E.: Sensitization to p-phenylenediamine. *A. M. A. Arch. Dermat.* 92: 276, 1965.

24. Spier, H. W.: Funktionelle Hautprüfungen bei allergischer Krankheiten in von Gottron, H. A., and Schönfeld, W.: *Dermatologie und Venereologie,* vol. 3, pt. 1, p. 393. Georg Thieme Verlag, Stuttgart, 1959.