Papulo-vesicular Count for the Rating of Allergic Patch Test Reactions

A Simple Technique Based on Polysulfide Rubber Replica

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Silicone rubber replica of patch test reactions were obtained from 12 patients with allergic contact dermatitis. 85 positive or doubtful reactions to allergens included in a standard battery were studied. The number of papulo-vesicles and sweat pores as seen in the replica were counted under a stereomicroscope. The papulo-vesicular count increased significantly in relation to clinical reading of the reactions of increasing strength. The number of sweat pores of positive reactions was decreased. This was probably due to cutaneous oedema with compression of sweat gland ducts. Papulo-vesicles are also consequences of the inflammatory oedema. The replica method was concluded to be a useful tool for the grading of allergic patch test reactions. It has the virtues of reproducibility, simplicity and low cost, and it can be evaluated under standardized laboratory conditions. Replica may also be the basis for more sophisticated methods of quantitation such as computer-aided image analysis. *Key words: Replica; Quantitation; Oedema; Papules; Vesicles; Sweat.*
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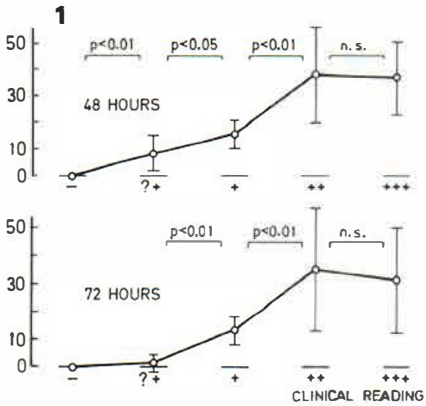
It is known that patch testing is not without hazard both with respect to the reading of positive reactions and with respect to interpretation and consequence (1). The International Contact Dermatitis Research Group proposed a standard terminology and criteria for the clinical reading (2). Later the criteria were specified with respect to erythema, papules and vesicles (3). Recently objective methods for quantification of the reactions were introduced. The new non-invasive methods include laser-Doppler flowmetry, evaporimetry, skin-fold recordings, and high-frequency ultrasound measurement of skin thickness (4, 5, 6, 7, 8, 9). However, these measures are comparatively expensive, and skill is needed for their proper use.

Silicone rubber replica have been used in dentistry for many years. Replica has been used in dermatology for the study of disturbed keratinization (10). The method is cheap and easy in use, and splendid imprints of surfaces are obtained. Previously we studied epidermal atrophy and sweat gland activity in localized scleroderma (11). In the present study we introduce the replica technique as a tool for the quantification of allergic patch test reactions.

MATERIAL AND METHODS

Twelve patients with allergic contact dermatitis according to history and previous patch testing were studied. Their mean age was 59 years (range 25–77 years). They were all tested with the ECDRG standard battery of contact sensitizers (22 common allergens) applied to the upper back for a 48-hour period using standard Finn ®chambers on Scanpore®. They showed a total of 85 positive or doubtful reactions. Clinical reading was done according to the guidelines of the International Contact Dermatitis Research Group (3). The reading was done by staff members of the skin test laboratory 48 hours, 72 hours and 7 days after application of the chambers.



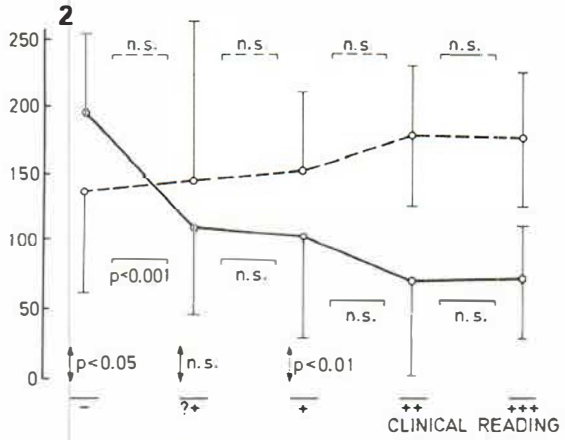


Fig. 1. Papulo-vesicular count of allergic patch test reactions after 48 and 72 hours. Horizontal markings indicate clinical reading. Values are expressed as mean values and standard deviations of observations. 0.64 cm^2 represents the test chamber area.

Fig. 2. Sweat pore count of allergic patch test reactions (−) in comparison with regional control of normal skin not exposed to test chambers (– – –). Examinations were performed 48 hours after application of test chambers. Horizontal markings indicate clinical reading. Values are expressed as mean values and standard deviations. 0.64 cm^2 represents the test chamber area.

Replica were taken from tested areas and normal skin of the same body region in relation to clinical reading. Replica were also taken from test areas without clinical reaction. A polysulfide silicon rubber imprint material (COE-flex® regular, Coe Laboratories Inc., Chicago, USA) was used. This material has a minimum working time of 3 1/2 min before curing. Replica were studied under a Zeiss stereomicroscope with magnification 9–25. The tested areas were divided into quadrants, and negative imprints of papules and/or vesicles were counted. The replica was illuminated with thirty-degree incident light from a halogen lamp. The object was rotated on the table to reach the angle, which gave an optimal presentation of the skin surface relief and the papulo-vesicles. The evaluation of the replica was performed by one of the authors (K. P.) without regard to the clinical evaluation done by the staff. Furthermore, sweat pores seen as distinct droplet-imprints were counted following principles previously described (11).

Statistical analysis was carried out by Student's *t*-test. *p*-values less than 0.05 were considered significant.

RESULTS

Fig. 1 shows papulo-vesicular counts at 48- and 72-hour readings. Papulo-vesicles were not observed in negative (−) test areas while doubtful, 1+ and 2+ reactions showed increased mean counts with significant differences between each group of reactions. 2+ and 3+ reactions showed non-significant differences. 3+ reactions were characterized by a heterogeneous relief with confluent vesicle-imprints or bullae, which could also sporadically be found in 2+ reactions. At the 7-day reading papulo-vesicles had widely disappeared except for 2+ and 3+ reactions, which still presented increased counts ($p<0.01$).

Fig. 2 shows sweat pore counts at the 48-hour reading. The pore count was significantly increased in test areas showing negative (−) reaction in comparison with normal skin, which had not been covered by a Finn® chamber. Doubtful reactions showed no significant difference, however, 1+, 2+ and 3+ reactions showed significantly decreased pore count in comparison with regional control of normal skin. Positive allergic reactions

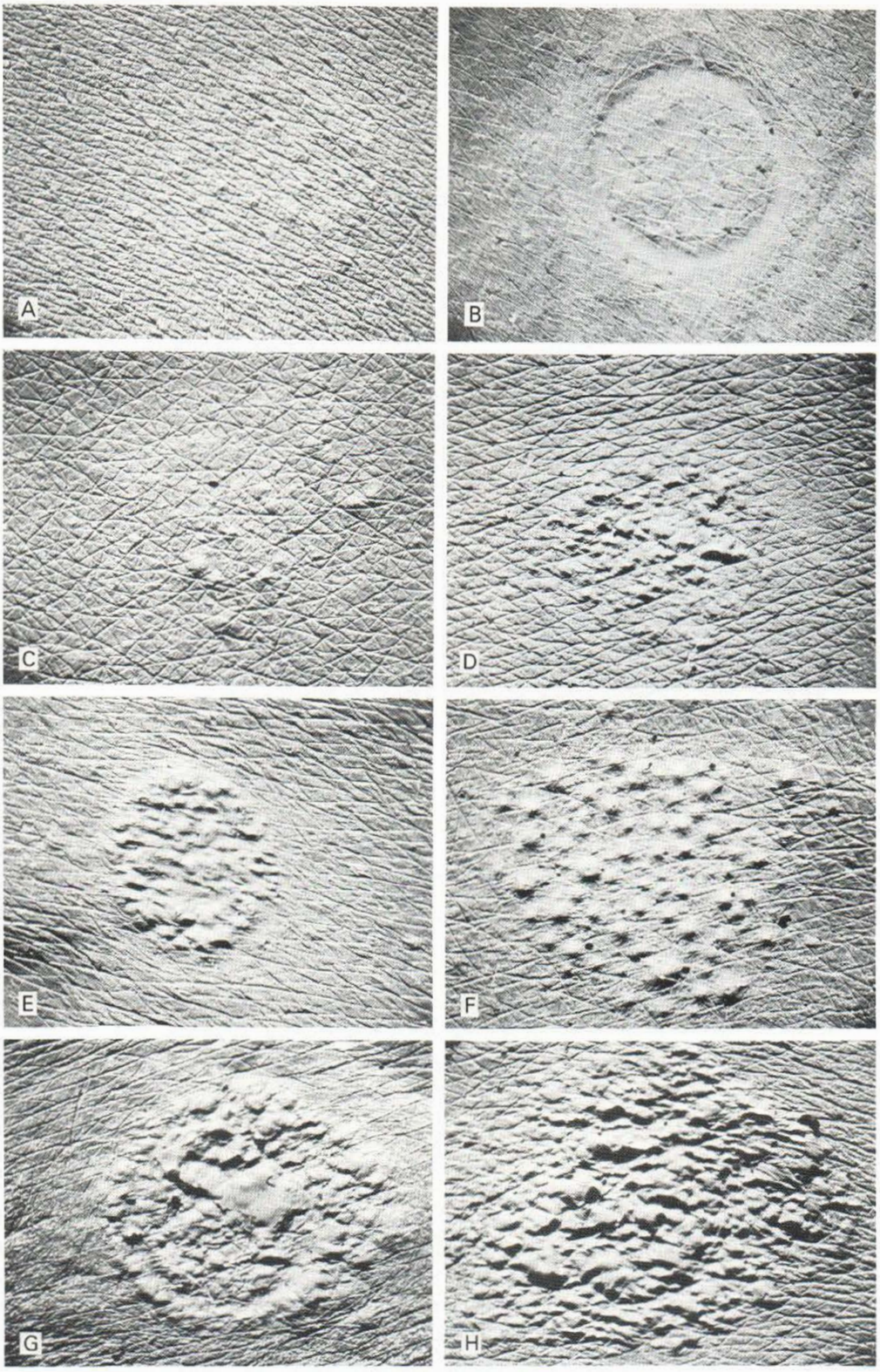


Fig. 3. Replica (photographically converted to positive) of ?+ reactions (A, B), 1+ reactions (C, D), 2+ reactions (E, F), and 3+ reactions (G, H).

Fig. 4. Replica of normal skin showing circular impressions of sweat droplets.

showed no significant differences between groups of different strength clinically. Thus, positive reactions could not be graded by the pore count, in contrast to the papulo-vesicular count. Curves of pore counts at the 72-hour reading also presented a significant decrease of positive reactions. At this time negative reactions showed no difference from regional control, and the increase in pore count of negative (−) reactions of the 48-hour replica previously mentioned was probably a result of a water-occlusive effect of the aluminium chamber. At the 7-day reading pore counts had normalized except for 2+ and 3+ reactions, which still presented decreased counts ($p<0.01$).

Repeated and blinded counting of 16 replica after 8 weeks showed the following mean values of the differences between values obtained at the two times: Papulo-vesicular count 0.5/0.64 cm^2 (SD 10.4); sweat pore count −0.8/0.64 cm^2 (SD 31.7), normal skin 14.6/0.64 cm^2 (SD 58.6).

DISCUSSION

The study shows that positive allergic patch test reactions can be graded by polysulphide rubber replica and papulo-vesicular count. Replica can be taken by relatively unexperienced personnel. They can be stored and sent by postage. For the evaluation only a low cost technical stereomicroscope is needed. Counting can be performed by a trained laboratory assistant. It is an advantage that the imprint evaluation can be done under laboratory conditions with full control of the illumination system and without problems with movements of the object etc. Due to these advantages the replica method is concluded a potential useful tool in clinical patch testing, and in particular for multicentric and

standardized evaluation. Live close-up photography is technically difficult and special set up and expertise is needed for a proper standardization and photographic quality.

Papulo-vesicles are, essentially, consequences of the inflammatory oedema of the reactions. Cutaneous oedema with compression of sweat gland ducts might explain the reduced number of sweat pores in allergic reactions. Reduced pore count might also be due to mediators of inflammation hampering the glandular production of sweat.

Reproducibility studies performed by the same observer after 8 weeks showed the papulo-vesicular and sweat pore counts of a group of samples to be reproducible, however, standard deviations indicated that counting of individual samples might be more variable. We did the countings without guiding from other observers and in an interrupted way, also carrying out the daily clinical work. Despite these difficulties the technique provided a meaningful and proper grading of allergic patch test reactions in comparison with a reference, i.e. clinical reading, which need not be true. With a concentrated counting procedure and the experience we have now, the intra-observer variation will probably be diminished. Observer problems in the counting are likely to be overcome by computer-aided image analysis or by more simple stylus or densitometric methods already described for the purpose of quantifying minute abnormalities of the skin surface as presented on replica (12, 13, 14).

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