

Effect of test techniques on perfusion of neomycin sulfate patch tests

A comparative study with visual assessments

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Effects of test techniques on neomycin patch test results have not been thoroughly investigated. This study focuses on effects of dose and application time of neomycin sulfate patch tests on test results. The effects are assessed both visually and by perfusion. 16 subjects positive to neomycin are retested with neomycin sulfate in various doses and vehicles applied with different application devices for variable time intervals. All subjects were tested with a serial-dose series for time intervals ranging from 3 to 96 h (4 days [D]). The results show very good agreement between perfusion assessments and positive and negative visual assessments of reactions; however, a gray zone was detected where reactions with variable perfusion were associated with visually questionable test responses. 1:27 serial dilutions and wide application time intervals show a positive response with perfusion assessments and partly with visual assessments. Regardless of reading technique, highest reactivity of reactions was detected at either 96 h (day [D] 4) or 168 h (D7) in each subject with minor exceptions, regardless of dose, vehicle, application device or application time. Some findings of the study support the idea that reactions with papules alone are positive tests. Possible effect of tapes and foils on test results are discussed.

Key words: allergic contact dermatitis; patch testing technique; tape; bioengineering methods; laser Doppler perfusion imaging; laser Doppler perfusion scanning; dose; application time; visual assessment; medicaments. © Munksgaard, 2000.

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Various effects that test techniques may have on neomycin sulfate patch test results are still unknown, despite the fact that neomycin should be ideal for such investigations because of its non-irritant properties (1). Traditional visual assessments and assessments of perfusion of patch tests are 2 methods available for such investigation. Where perfusion assessments are concerned, the alternatives are 2 different laser Doppler perfusion technologies, both of which are based on the same laser Doppler principle (2). One of these is called flowmetry and the other imaging or scanning. Guidelines regarding the use of each of these techniques have been proposed (3, 4). Flowmetry has been used for a long time, while the imaging technique is newer. Even though flowmetry is non-invasive, it requires the use of a probe that directly touches the skin during readings. The extent to which such touch may affect perfusion assessments is unknown. This problem is solved by imaging, which uses a scanning laser beam located in a laser head above the skin (5). With the help of 2 step-

ping motors, the laser beam is moved from 1 skin measurement site to another within the scanned area. At each site, assessment of perfusion is made by a computer system that bases its assessments on light reflected from the skin to a detector in the laser head. When a selected area has been scanned, its perfusion is shown as an image on a monitor. Such images are composed of all the measurement sites of each scan, where each measurement site is given a color representing a certain perfusion interval in arbitrary units. The current study uses the imaging technique for perfusion assessments. We have used such imaging before in other studies on patch tests (6–12). It has also been used by other investigators for patch test assessments (13–15) and for other applications (16–19).

The laser Doppler imaging is performed in current study with a laser Doppler perfusion imager® (LDPI). We use it as we have suggested for patch test assessments when non-pigmented test substances are tested on light skin (4). We have the following objectives with our study population.

(A) To see whether it is possible to use the LDPI to assess neomycin sulfate patch tests. (B) To investigate the agreement between LDPI and visual assessments. (C) To see whether neomycin sulfate patch test doses or application times may affect test results.

Subjects, Materials and Methods

Study populations

A random selection was made from files of subjects who were positive to no more than 2 patch tests when tested with the Swedish patch-test series, 1 patch of which was 20% neomycin sulfate in pet. (Chemotechnique Diagnostics AB, Malmö, Sweden). 16 white subjects participated, 13 women and 3 men (men nos. 2, 5 and 7), aged 24–61 years (median age 39). The files were obtained from 2 departments of dermatology and 1 department of occupational dermatology in Stockholm. The minimal visible tissue change for a positive test had been erythema and infiltration at the time of assessment. All subjects had been tested with 48-h (2-day [D]) applications with Finn Chambers® on Scanpor® tape (Epitest Ltd Oy, Tuusula, Finland) (20). Readings had most often been performed 24 h (1D) and 120 h (5D) following detachment of applications. 8 subjects had skin type II (subjects nos. 1, 7–9 and 12–15), 5, type I (subjects nos. 3, 4, 6, 11 and 16), 2, type III (subjects nos. 5 and 10) and 1, type IV (subject no. 2). 6 subjects had clinical relevance between their allergy and a product containing neomycin (subjects nos. 3, 5, 9, 11, 13 and 16) while the others were uncertain. The intensities of reactions were unknown to the investigators prior to and during the study. Subjects were asked to report reactions appearing after the study. No such reports were received in the months following the study.

Exclusion criteria

These were medication or disorders known to affect perfusion or allergic reactions in general, recent or current treatment with ultraviolet radiation, high risk of a patch-test location over a pathological skin condition, atopic disease, or patch testing or a history of eczema within the last 2 months. None of the subjects were smokers. All subjects were asked not to consume alcohol or caffeine during the study.

Application materials

Test materials are shown in Table 1. All serial patches containing 0.23 mg/cm² neomycin sulfate or a lower dose contained a methylcellulose vehicle and were chemically analyzed (Pharmacia A/S, Hillerød, Denmark). The 0.69 mg/cm² patch was also chemically analyzed but contained a polyvidone vehicle. The 0.4 mg/cm² patches were of our own manufacture and contained a carboxymethylcellulose vehicle and an approximated dose of neomycin sulfate. The 20% neomycin sulfate test substance in a demineralized water vehicle was also of our own manufacture while the one containing the petrolatum vehicle was supplied by Chemotechnique Diagnostics AB, Malmö, Sweden. Test areas were 9×9 mm with the polyester patches and 9 mm in diameter with the Finn Chambers® (Epitest Ltd Oy, Tuusula, Finland). Each of the tests applied directly by foils was located within a 9×9 mm area.

2 control patches were applied with each application strip. These were identical to patches containing allergens, with the exception that carboxymethylcellulose was used for control purposes for all cellulose-containing patches. Demineralized water was used to control the allergen test substance containing it, while petrolatum-containing patches

Table 1. Test applications on subjects

Patches	Application device	Population tested
serial doses containing approximately 0.4, 0.23, 0.0766, 0.0255 and 0.0085 mg/cm ² n.s. printed on polyester squares	1 patch of each dose applied by both foils and tapes	all subjects except for the approximately 0.4 mg/cm ² n.s. patches which were tested only on subjects nos. 8–16
20% (w/w) n.s. in petrolatum applied in Finn Chambers®	tape	all subjects
20% (w/w) n.s. in petrolatum	foils to which the test substance was directly applied	subjects nos. 8–16
20% (w/w) n.s. in demineralized water	foils to which the test substance was directly applied	subjects nos. 8–16
0.69 and 0.23 mg/cm ² n.s. printed on polyester squares	1 patch of each dose applied by a tape strip	only subject no. 8

n.s.: neomycin sulfate.

were controlled with white petrolatum (Apo-teksbolaget, Gothenburg, Sweden). All tests applied directly by foils or in Finn Chambers[®] contained approximately 20 μ l of the test substance.

Non-woven cellulose acetate tapes with acrylic adhesive were used for tape applications (Ceder-roth BV, Tilburg, The Netherlands). Foils were Tegaderms[™], which are thin, adhesive and semi-occlusive (3M Svenska AB, Sollentuna, Sweden). Tape sizes ranged from 5×6–12 cm and foils were 10×12 cm in size. Patches were located randomly on each tape strip or foil in an order unknown to the investigators. Patches of tape strips and all foils not containing a 20% test substance were applied in 2 straight, parallel columns 1 cm apart and with a 2 cm distance between patches of each column, forming a staggered pattern between the columns. Patches of foils with the 20% test substances were applied in 5 rows separated by 1 cm with a 2-cm distance between patches of each row. The patches of each row alternated between 2 and 3 so that adjacent columns formed a staggered pattern.

All application materials were kept for 4 days following preparation in a refrigerator at 4–8°C. They were refrigerated in air-tight and light-impermeable envelopes until the application, except for the aqueous and petrolatum substances which were applied directly from the refrigerator to foils or Finn Chambers[®] only a few minutes before being applied on subjects.

Applications on subjects

Applications on subjects are shown in Table 1. All tape strips and foils were randomized before being applied vertically on subjects' backs. Each subject was tested with the strips with the serial doses for 3, 6, 24 (1D), 48 (2D), 72 (3D) and 96 h (4D), with the Finn Chambers[®] for 48 h (2D) and with a foil for 168 h. The strip with the 0.69 and the 0.23 mg/cm² patches was applied separately for 48 h (2D) approximately 2 months following application of the other test materials. Some patches had to be removed before planned because of discomfort (Finn Chambers[®] and the 96-h (4D) application strips on subject nos. 1 and 16, and the 72-h (3D) strip on subject no. 16).

Readings

Reading sessions were at 3, 6, 24 (day [D] 1), 48 (D2), 72 (D3), 96 (D4), 168 (D7) and 216 h (D9) following the application. Tests of subject no. 1 and nos. 8–16 were read additionally at 240 h (D10), and at 264 h (D11) on all other subjects. During each reading session, all patch test areas not covered by application devices were read ex-

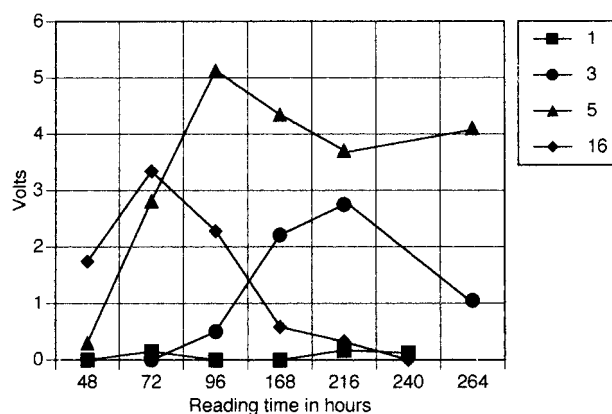


Fig. 1. Adjusted perfusion profiles of subjects tested for 48 h (2D) with 0.0255 mg/cm² neomycin sulfate polyester patches on tape.



Fig. 2. Foil application area approximately 30 min after detachment of the foil. Please see text.

cept for a single reading at 72 h (D3) of tests of the strip with the 0.69 and the 0.23 mg/cm² neomycin patches. Patch tests applied with foils were also read at each session prior to their detachment and

2× at the session when they were detached, both prior to and following their detachment.

Reactions were first read with the LDPI and then assessed visually using a standard accepted by the North American Contact Dermatitis Group (21). Approximately 11,700 readings were performed. Up to 2.5 h were required for each of more than 160 subject sessions.

Labels attached outside tape or foil borders helped relocate test areas after detachment of strips or foils. In most cases, at least 30 min passed between detachment of an application material and readings, or between a subject's arrival and readings.

Subject-related and environmental factors

Ultraviolet radiation treatment and sunbathing were prohibited, as was medication known to affect pigmentation, perfusion, or the immune system. Body back temperature ranged from 29–33°C.

LDPI readings

Perfusion was read and analyzed with a laser Doppler perfusion imager® PIM 1.0 (LDPI), using LDI version 2.5 software (both supplied by Lisca Development AB, Linköping, Sweden). The instrument set-up has been published (4). Highest perfusion is 10.00 V; lowest, 0.00 V. The laser beam was focused at a distance of 17 cm between the laser head aperture and test areas. Applied Laser Pen® LP 30 (Applied laser systems, Grants Pass, OR, USA) was used to secure an exact reading distance, as used before (4).

Assessment of perfusion

Mean perfusion value is calculated for each of 2 identical control patches and subtracted from the corresponding value of the allergen-containing patches tested on the same strip or foil. Such perfusion is termed "adjusted perfusion", and its value, "adjusted perfusion value" (APV), indicated in V.

Ethical approval was obtained from the Ethical Committee of the Karolinska Institute, Stockholm (No. 95/130).

Results

Perfusion assessment of patch tests

The LDPI may be used for assessment of neomycin sulfate patch test reactions. The instrument is sensitive enough to assess inter-individual variation in perfusion with identical tests applied equally long. The follow-up of test areas over time makes it possible to chart perfusion in terms of APVs at desired time intervals. This is demonstrated in Fig. 1 for some subjects where perfusion profiles may be compared.

Agreement between LDPI and visual assessments

The agreement between highest visual and highest APV assessments of reactions is shown in Table 2. For a reaction to be included in the analysis, its highest reactivity must be captured during the observation time with both assessment methods. 2 kinds of disagreements were observed.

(A) Negative reactions with APVs of ≥ 0.9 V. The 3 negative reactions in Table 2 with APVs of ≥ 0.9 V have APVs of 2.21, 1.59 and 0.94 V. The 2.21 V value resulted from a reading of a patch applied with foil, with the reading performed shortly after Fig. 2 was taken, approximately 30 min following careful detachment of the foil. The location of the patch in the figure is where a vertical line drawn from the left of the 2 top label markings in the figure crosses a horizontal line drawn from the uppermost marking of the 3 label markings on the left. Before detachment, the perfusion of the patch was 0.49 V, but 4.41 V following detachment (APV of 2.21 V). Upon detachment of the foil, the entire foil-application area became highly erythematous and perfusion of all allergen-containing patches and control patches increased. In the study population, such increased perfusion was observed in 72% of control patches and 83% of serial patches, with 6 of 16 subjects increasing perfusion of all patches and surrounding skin, while none had decreased perfusion of all patches. During the reading session, the erythema declined. The APV of 2.21 V resulted from a test with the 3rd highest serial dose. That subject even reacted with erythema of patches and skin exposed to

Table 2. The relationship between visual and APV assessments of highest reactivity of reactions

Visual assessment	No. of reactions with APVs <0.9 V	No. of reactions with APVs ≥ 0.9 V	Median APV (V)	APV range (V)
—	171	3	0.11	0.00–2.21
?/?+	41	17	0.36	0.00–3.34
+	1	19	2.11	0.47–4.34
++	0	87	2.78	0.95–5.85
+++	0	32	4.895	2.16–5.85

long-time application strips, with declining perfusion of control patches during the session when strips were detached.

The APVs of 1.59 and 0.94 V with negative tests in Table 2 are 96 h (4D) readings of 72 (3D) and 48 h (2D) tape applications, respectively, on a different subject than the one with the APV of 2.21 V above. Both APVs result from tests with the 3rd highest serial dose tested on that subject. Following detachment of the application tapes of these patches, irritation by the test tapes surrounding the patch test areas was observed despite careful detachment of strips. It persisted until at least the 168 h (7D) readings. The 1.59 V value resulted from increased perfusion at the allergen patch test area at 96 h (D4), at the same time as perfusion of an adjacent patch test area with the lowest dose increased. The other APV resulted from increased perfusion at 96 h (D4), as for all other patches including control patches of that strip, except for the 2nd highest serial dose (0.23 mg/cm²) located in the corner of the strip.

(B) A positive reaction with an APV of <0.9 V. The only positive reaction in Table 2 with an APV of <0.9 V is a + reaction resulting from a foil

application of the highest serial dose tested on that subject. That reaction becomes positive at 216 h (D9), but has the highest APV of 0.47 V at 168 h (D7) prior to detachment of the foil. Readings taken at least 30 min following detachment of the foil showed decreased perfusion of all patch test areas, including control patches, except for the weakest serial dose where perfusion was slightly increased.

Effect of test doses on reactivity

Table 3 shows the effect of changes of adjacent serial doses on reactivity. All comparisons of reactivity between doses in the table with each reading method only consider tests of doses where visible reactivity is observed, and doses where a positive reaction is compared to a – or a ?/?+ test. Higher reactivity in the table with decreased dose cannot be explained by only a few subjects.

When comparing test results of serial doses that are far apart from each other to those that are next to each other, the results generally show an increased number of reactions with lower rather than higher APVs in relation to decreased dose, when

Table 3. Effect of dose changes on reactivity

		Change in doses (from → to)							
		1 → 2	2 → 3	3 → 4	4 → 5	1 → 2	2 → 3	3 → 4	4 → 5
		perfusion assessment (APV)				visual assessment			
		tape applications							
3	↓	0	1	0	0	0	1	0	0
	↑	0	0	0	0	0	0	0	0
	—	0	0	0	0	0	0	0	0
6	↓	0	3	3	3	1	1	2	2
	↑	1	2	1	0	0	2	0	0
	—	0	0	0	0	0	1	1	0
24	↓	1	4	7	4	1	1	3	0
	↑	2	3	0	1	0	0	0	0
	—	0	0	0	0	2	6	2	3
48	↓	3	6	5	5	1	3	3	3
	↑	0	2	2	1	0	0	1	0
	—	0	0	0	0	3	5	3	3
72	↓	2	4	6	5	2	1	2	3
	↑	1	3	1	1	0	1	0	0
	—	0	0	0	0	1	5	5	4
96	↓	1	3	3	4	1	0	0	0
	↑	0	1	1	0	0	1	0	0
	—	0	0	0	0	0	4	3	4
		foil applications							
168	↓	3	4	4	6	4	1	1	3
	↑	1	4	3	1	0	1	0	0
	—	0	0	0	0	1	7	7	3

Numbers show number of reactions. For inclusion criteria in the table, please see text. Serial doses of neomycin sulfate (mg/cm²): no. 1: approximately 0.4; no. 2: 0.23; no. 3: 0.0766; no. 4: 0.0255 and no. 5: 0.0085.

↑: Increased reactivity; ↓: decreased reactivity; –: unchanged reactivity.

comparing the results of tests of different doses but the same vehicle (from 0.23 to 0.0085 mg/cm²). When comparing results of tests with the 0.23 mg/cm² and the lowest serial dose of 0.0085 mg/cm², all reactions yield lower APVs at the lower dose. With visual assessments, all comparisons of widely separated serial doses to those that are next to each other yield lower or unchanged intensity with decreased dose. However, when test assessments with the 20% test substance, which contains approximately 5.35 mg/cm² neomycin sulfate, are compared with test assessments with either the approximately 0.4 or the 0.23 mg/cm² doses, some tests have higher APVs at these lower doses. This is also the case when visual assessments of reactions with the approximately 5.35 and the 0.23 mg/cm² doses are compared.

Effect of application times on reactivity

Reactions are detected with all application times, but with 3-h applications only a single reaction is detected on a single subject. The effect on reactivity of tests caused by a change in application time between adjacent intervals is shown in Table 4. All comparisons of reactivity between application times with each reading method only consider tests of application times where visible reactivity is noticed, and tests of application times where a positive reaction is compared to a – or a ?/?+ test. The effect of applications on results is less clear in the table when applications are 24 h (1D) or longer with LDPI assessments and 6 h and

longer with visual assessments, where many reactions are stronger at the shorter than the longer application. This result is not explained by a few subjects reacting differently from others.

When comparing test results of wider application time intervals to those next to each other in Table 4, the general rule is that relatively more tests increase than decrease in intensity with extended application times, regardless of reading methods. If the interval is extended for 2 days, the number of tests that increase in intensity is higher where differences in results of 48 (2D) and 96 h (4D) applications are concerned, compared to results when the interval is extended from 24 (1D) to 72 h (3D).

Tests with papules alone

Some tests developed only papules, of which the following is of interest:

With the 6 h application and visual assessments, 2 application strips develop a positive reaction where only a few papules develop at an adjacent higher and 2 lower doses. Such a comparison between tests with papules only and positive tests are not made at longer applications in Table 3, where mostly positive tests of different intensities are being compared.

Test results for a subject, when tested with 0.23 mg/cm² neomycin sulfate (dose no. 2) in Table 4, show decreased visible test response when the application time is extended from 6 to 24 h (1D). With the 6-h application, that subject had a + re-

Table 4. Effect of changes of application times on reactivity

Dose	Reactivity	Perfusion assessment (APV)					Visual assessment				
		change in application time in hours (from → to)									
		3 → 6	6 → 24	24 → 48	48 → 72	72 → 96	3 → 6	6 → 24	24 → 48	48 → 72	72 → 96
1	↑	0	3	4	0	2	1	2	2	0	0
	↓	1	0	0	2	0	0	0	1	0	0
	–	0	0	0	0	0	0	1	1	2	0
2	↑	3	6	4	5	2	3	5	2	0	2
	↓	0	0	4	2	2	0	1	0	2	0
	–	0	0	0	0	0	0	1	6	4	3
3	↑	3	7	1	7	3	4	4	4	2	2
	↓	0	0	7	0	2	0	0	2	2	0
	–	0	0	0	0	0	0	2	2	3	3
4	↑	2	3	5	4	4	2	3	3	1	2
	↓	0	1	2	3	2	0	0	1	1	0
	–	0	0	0	0	0	0	1	1	5	4
5	↑	0	3	1	3	5	0	4	1	1	4
	↓	0	0	3	1	1	0	0	2	1	0
	–	0	0	0	0	0	0	0	2	2	2

Numbers show number of reactions. For inclusion criteria in the table, please see text. Serial doses of neomycin sulfate (mg/cm²): no. 1: approximately 0.4; no. 2: 0.23; no. 3: 0.0766; no. 4: 0.0255 and no. 5: 0.0085.

↑: Increased reactivity; ↓: decreased reactivity; –: unchanged reactivity.

action, with the 24-h (1D) application, a reaction with only some papules, and with the 48-h (2D) application, a +++ reaction.

With the visual assessment approach, 12 changes of reactivity show a decrease with extended application above 24 h (1D) in Table 4. 4 of these comparisons occur where a reaction is positive with the shorter application, but where only papules are observed with the longer application.

7 subjects developed reactions with papules alone with 3-h applications, but ++ or +++ reactions with longer applications of identical patches and application device.

Such reactions were not observed at short applications while longer applications are negative.

Early test reactivity to a neomycin sulfate patch test is shown in Fig. 3. The APV of the test is 1.33 V, while the mean value of corresponding control patches is 0.19 V.

Time point of highest reactivity

An increase in perfusion for reactions was generally observed before a visual test response was detected. Such observations are, however, subject to some uncertainty concerning the time of initiation of some of the visible test responses. Reactions with early high APVs did not necessarily yield higher peak APVs than reactions starting later.

Regardless of test techniques, highest APVs and visual assessments of reactions were detected on the same subject at the same reading time, with minor exceptions for the next time interval. The time points were at either 96 (4D) or 168 h (7D), except in 1 subject where it was at 72 h (3D). The time consistency of highest perfusion may be seen for a subject in Fig. 4 at 96-h (4D) readings of reactions elicited with 2 different doses and application times.

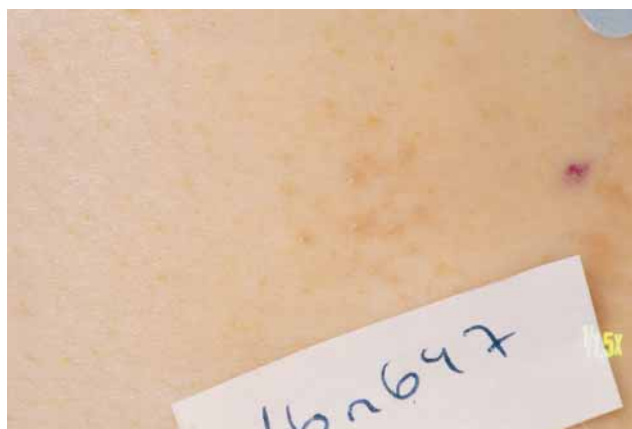


Fig. 3. An early patch test response to neomycin sulfate.

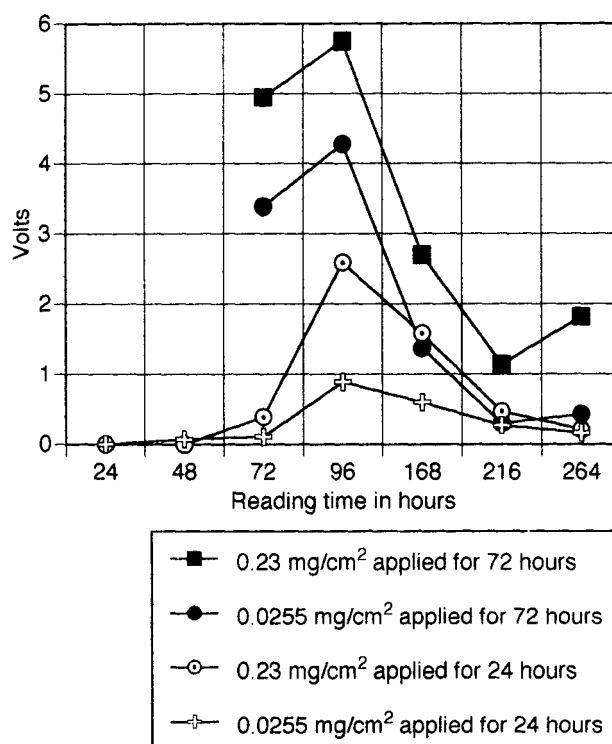


Fig. 4. APV perfusion profiles resulting from tests with 2 different doses of neomycin sulfate and application times of tape strips.



Fig. 5. Reaction of subject no. 8 tested with 0.69 mg/cm² neomycin sulfate for 48 h (2D).

Special tests on subject no. 8

The 48-h (2D) application of the 0.69 mg/cm² neomycin sulfate polyester patch resulted in an APV of 0.61 V and visually a + reaction when read at 72 h (D3) (single application and reading). The reaction is shown in Fig. 5. Concomitant testing with the 0.23 mg/cm² neomycin sulfate patch on the same application strip yielded an APV of 0.01 V and a ?/?+ response, while the initial testing with

that dose was negative. On the same subject, a Finn Chamber[®] test, also applied for 48-h (2D), with the 20% test substance in petrolatum (approximately 5.35 mg/cm² neomycin sulfate), resulted in the highest APV of 2.13 V but visually a ??+ test response that morphologically consisted of a few discrete papules.

Discussion

Assessment of perfusion

This study demonstrates that the laser Doppler perfusion imaging technique appears to be a good method for objective and non-invasive assessment of perfusion of neomycin sulfate patch test reactions. In earlier studies, we have even found the technique to be helpful for assessment of patch tests with other allergens; nickel sulfate hexahydrate (7), methylchloroisothiazolinone/methylisothiazolinone (8), budesonide (10, 11) and Myroxy-lon Pereirae (12). In these studies, we have shown that the LDPI may be used to assess inter-individual variation of perfusion of identical patches applied identically and equally long. The same is also true for neomycin sulfate patch tests as may be seen in Fig. 1.

Agreement between the reading techniques

One disadvantage with visual assessments of patch tests is that they are subject to readers' knowledge and experience (22). This may be solved by objective assessments as made possible with the laser Doppler perfusion imaging technique. The agreement between these 2 assessment approaches in Table 2 is promising when the number of reactions with APVs of <0.9 V or ≥0.9 V are matched to visually negative and positive reactions of variable reactivity grades. The 3 negative reactions that have APVs of ≥0.9 V and the single positive reaction with an APV <0.9 V comprise only 1% of visually positive and negative reactions in the table.

58 of 371 reactions (16%) are in a gray zone with ??+ visual assessments associated with a wide range of APVs. This group of tests interferes with the possibility of dividing reactions into 2 groups, those visually negative with APVs <0.9 V and those visually positive with APVs ≥0.9 V, with then only 1% of reactions not fitting the classification. Such a gray zone has not been our experience with other allergens, where we have observed good or generally good agreement between APVs and visual assessments of patch tests of nickel sulfate hexahydrate (7), Myroxy-lon Pereirae (12) and budesonide (10), and a moderately good agreement with methylchloroisothiazolinone/methylisothiazolinone patch tests (8).

Effect of foils on perfusion

2 of the badly fitting APVs with visual assessments result from foil applications. As the 2.21 V APV associated with a negative test results from the 3rd highest serial dose tested with the foil on that subject, irritation is a likely explanation for that high APV. This may not be the case for the 0.47 V APV associated with a + test, as all lower doses tested with the foil on that subject resulted in lower APVs and negative tests.

While some immediate erythema and increased perfusion may be expected following detachment of foils (Fig. 2), decreased perfusion is observed in some cases. An increase may possibly be explained by trauma caused by detachment of foils, if tightly adherent to the skin, supported by visible erythema of foil-application areas following detachment of foils. Another possibility for increased perfusion is that tight adherence of foils may press polyester squares against the skin, causing decreased perfusion which is relieved upon detachment of squares. Variable trauma due to the detachment and/or variable pressure of squares may explain inter-individual difference in perfusion following detachment of foils. Foils are interesting as they make possible assessment of perfusion of patch tests while applied to the skin (7, 12); and do not seem to affect perfusion in a study with irritant patch tests (15). Even though foils may not allow assessment of the effect their detachment may have on perfusion, they are of interest as they make possible assessment of perfusion without interference due to detachment of patch test application devices such as tapes. Regardless of how perfusion of patch tests changes immediately following detachment of foils, clearly an acclimatization time should be provided between detachment of foils and test readings.

Effect of tapes on perfusion

The 2 high APVs of 1.59 and 0.94 V associated with negative tests may be due to irritation. Supporting this is: (i) that these 2 APVs are higher than the ones resulting from 2 higher doses tested on both strips; (ii) that both these APVs are detected at the same reading (96 h); and (iii) that no such high APVs were associated with negative tests when irritation of strips was not observed. In favor of a localized irritation affecting the 1.59 V value is that perfusion of an adjacent lowest serial dose level is the only other test area which increases in perfusion at 96 h (4D). Supporting the effect of irritation on the other value is increased perfusion of all patches, including control patches, except for

the 2nd highest serial patch which is tested in the corner of the strip.

Irritation of application tapes may be clinically very important for patch tests, as shown in a recent study with the LDPI, where different brands of application tapes affected results of irritant patch tests (9). It is interesting that high APVs associated with negative tests were not observed when tapes did not irritate the skin. It may indicate that perfusion of patches may increase once there is inflammation in the vicinity of test sites, as in the excited skin syndrome (23–25). The effect of tape-strip irritation on test results is further supported by the perfusion values of the control patches of long-time application strips on the subject where the perfusion declined during the session when strips were detached. In fact, the effect has been repeated in a recent study on that same subject tested for 72 h (3D) with identical patches and tape as used in current study (4).

Effect of test doses on reactivity

In Table 3, intensity of many visual assessments is unaffected by dose, while some assessments reveal decreased intensity with decreased dose, as is the case with the majority of the perfusion assessments. A possible explanation for why so many visual assessments are unaffected by dose in the table may be that some changes in intensity are simply not enough to affect the reactivity grade on the narrow assessment scale used for visual assessments of positive tests (+ to +++). When perfusion assessments in the table are studied, not a single pair of compared tests is of the same intensity. This is because the perfusion assessment scale is much more precise than the one used for visual assessments (0.00 V–10.0 V).

26% of perfusion assessments and 6% of visual assessments in Table 3 show higher reactivity when the dose is decreased. The high % of such results with the perfusion is partly explained by the effect that very little difference in perfusion between reactions of adjacent dose levels may have, if the lower dose yields slightly higher APV than the higher. The difference in the %s between the assessment methods is at least partly explained by the precision of the assessment scales, as minor changes in reactivity may be rendered of unchanged intensity by the visual assessment scale.

While some reactions are stronger with lower dose when results of each of 1:3 serial dilutions are compared in Table 3, this is not the case when a 1:27 dilution is used; 0.23 versus 0.0085 mg/cm² (same vehicle). It is interesting that a 1:23 dilution with much higher doses, 5.35 (20% substance) versus 0.23 mg/cm², does not give results similar to

the 1:27 dilution. It may indicate just decreased response or same visible response in relation to decreased dose only when low doses are compared. This may seem to be supported by the + reaction with the 0.69 mg/cm² dose on a subject with a reaction of only a few papules when tested with approximately 5.35 mg/cm² dose in a Finn Chamber[®] (20% substance), with multiple readings and checks of chamber contact. However, it must be remembered that different test techniques are being compared and real bioavailability of the allergen is unknown.

Effect of application time on reactivity

Only a single reaction of a single subject is positive with 3-h applications. This suggests that a certain period of contact by the active test agent is necessary for elicitation of reactions. Our experience with methylchloroisothiazolinone/methylisothiazolinone patch tests is different than with neomycin, as with the former all subjects tested with 6-h applications elicited positive tests (8). It is possible that neomycin test materials other than those used here may make short applications more efficient.

Almost all comparisons of reactivity show increased reactivity with extended applications from 3 to 6 h or from 6 to 24 h (1D), where perfusion is concerned, and all when the time is extended from 3 to 6 h, where visual assessments are concerned (Table 4). The relationship is still good when the application time is extended from 6 to 24 h (1D) with visual assessments, as 18 assessments show increased response with extended time, 5 are unchanged, while only 1 comparison shows a decreased response. The observation that more tests increase in intensity when 48- (2D) and 96-h (4D) versus 24- (1D) and 72-h (3D) application-time intervals are compared may suggest increased allergic reactivity with very long applications; however, irritation by longer contact of strips cannot be excluded.

Tests with papules only

The morphology of neomycin sulfate patch tests may have a special character, as can be seen in Fig. 5. Histopathological examination of positive neomycin sulfate patch tests may mainly show an inflammatory infiltrate in the dermis centered around sweat ducts and hair follicles (26). Vascular dilatation and oedema in the dermis has also been noted (27). Epidermal changes may be minor and become more pronounced with time (26). Supporting dominant dermal reactivity in the current study are papular eruptions of test responses but not vesicular lesions (26). The use of a high-fre-

quency ultrasound, or assessment of superficial perfusion with a laser light of a shorter wavelength than used here, may be of help in assessing the level of dermal reactivity. However, some authors consider neomycin sulfate patch tests to have a strong epidermal component (27, 28) and it has been suggested that the ratio between dermal and epidermal elements may vary enormously between cases (27). The morphology of neomycin patch tests with papules alone and tests assessed as $+/+$ in the gray zone with high APVs, in current study, may suggest that perfusion assessments may be better suited than visual ones to assess patch tests. Obviously, for positive reactions, some other reference than visual assessments is required for interpretation of perfusion of such tests, such as patient histories or still other assessment techniques.

Weaker test responses than shown in Fig. 3 may be overlooked by some readers or assessed as perifollicular reactivity not related to allergy. Visual assessment scales may be convenient to apply in practice but must only be considered as guidelines, as a single scale may not suit assessments of all tests regardless of allergens and test techniques. This must be kept in mind when neomycin sulfate patch tests are assessed because of the special morphology that may be involved (Fig. 5). The morphology may not fulfill criteria for a positive test with the assessment scale used in the current study, which may explain the number of tests in the gray zone in Table 2 with $+/+$ test responses but high APVs. Awareness of this problem with the visual assessment may be a reason why authors have designed special scales in their studies for the assessment of neomycin patch tests (29–31). Criteria for positive tests may differ between scales. For example, one scale requires neither erythema nor infiltration for a positive test (30), both of which, in addition to discrete papules, are required by the scale used in current study (21). With the former, few papules alone are sufficient for a test to be positive (30). Such reactivity would be regarded as negative with a scale used by another author, regarding a few, scattered, small, nonconfluent papules as a negative reaction, and requiring confluent papules for a positive reaction (29, 31). By that scale, the reactivity of the patch test in Fig. 3 would thus be regarded as negative. In favor of that reaction really being an allergic reaction is an APV of 1.33 V, a change in morphology on follow-up to erythema and infiltration and clinical relevance between the subject's allergy and a product containing neomycin.

Both tests of different doses and application times show that tests with papules alone are indeed positive reactions. In favor of positive tests is that: (i) such reactions are observed at patches tested on

the same test strip at both higher and lower doses than the one yielding a positive test; (ii) they may be found at both a shorter and a longer application than a positive test, all tested with an identical patch on the same subject; (iii) such reactions are detected at a long application, while a shorter one with an identical patch yields a positive test in 4 cases; (iv) they are found only at the shortest application of only 3 h in 7 subjects, while $++$ or even $+++$ reactions are elicited with longer applications of identical patches and application device; (v) they are not found with short applications while longer applications of identical patches are negative. Perfusion assessments also suggest that such reactions are positive, as in one subject such a reaction with an APV of 2.13 V is observed with the 5.35 mg/cm² dose while with the 0.69 mg/cm² patch a $+$ reaction is elicited associated with an APV of 0.61 V.

Time point of highest reactivity of reactions

It is interesting that highest reactivity of reactions is generally detected at the same, or at almost the same, reading times with both reading methods. This was not our experience with nickel sulfate (7) or methylchloroisothiazolinone/methylisothiazolinone (8), where the perfusion generally reached its highest value before the visual assessment. For neomycin sulfate, that the highest value was detected at the same time with both reading techniques may be explained by either a slower increase in perfusion, or an earlier visible tissue change compared to the other allergens. Our experience with budesonide showed a tendency similar to that of neomycin sulfate (10), but with Myroxyton Pereira we detected highest reactivity of reactions with both methods at the same time or first with the visual assessment approach (12). These findings reflect a different time factor between highest perfusion and strongest morphological response to patch tests with different allergens.

Interestingly, different test doses, application methods, and application times resulted in the same or nearly the same time point of highest reactivity with both reading techniques for the same subject, as can be seen with the 2 different doses and application times in Fig. 4. We have generally found little or no change of the timing in relation to doses or application times with patch tests of 3 different allergens; nickel sulfate hexahydrate (7), budesonide (10) and Myroxyton Pereira (12). However, with methylchloroisothiazolinone/methylisothiazolinone patch tests, we found an unpredictable timing in relation to a change either in application time or of dose (8).

Inconsistent elicitation of test responses

A problem with neomycin sulfate patch tests is that positive test responses may be inconsistent (26). Even though not the issue with current study, this was the case with the only retesting that was carried out, as on the 2nd testing the patch test yielded a $+/+$ test response at a single reading (72 h (3D)), while multiple readings of the 1st identical test were negative (0.23 mg/cm² patch). The reason for the inconsistency is unknown but it must be kept in mind that these 2 tests were not performed concurrently.

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