Revised Open Application Tests (ROAT) in Patients Allergic to Colophony – Evaluated Visually and with Bioengineering Techniques

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It is desirable to further evaluate the clinical relevance of a positive patch test. The repeated open application test (ROAT) has been suggested as such a supplementary method. To compare the results of patch testing with the outcome of ROATs, 13 colophony-sensitive subjects and 9 controls were patch-tested with colophony in a serial dilution test. Five microlitres of three concentrations of a colophony solution and the vehicle were then applied to small test areas on the lower arm, once daily for 2 weeks. Prior to each application, all test sites were examined visually and with bioengineering techniques.

In the ROATs, 10/13 colophony-sensitive subjects – but no controls – reacted to a 20% colophony solution, 4 also to 1%. A correlation was found between the threshold concentration at patch testing and the outcome of ROATs. There was great variation in the reactivity in the ROATs. Objective measures for evaluating the ROAT reactions gave no further information than visual assessment. Key words: laser Doppler flowmetry; serial dilution patch testing; sodium lauryl sulfate; threshold of sensitivity; transepidermal water loss.

The clinical relevance of a positive patch test reaction is sometimes difficult to evaluate (1). In previous studies we have found positive patch test reactions to colophony in individuals with no ongoing dermatitis, despite colophony exposure (2–4). It therefore seems important to find other ways of evaluating the clinical relevance of a positive patch test reaction. Use tests, e.g. “the repeated open application test”, ROAT (5), often imitate every day exposure to an allergen better than a single patch test does. In clinical practice, the ROAT has so far been used mostly for formulated products. There are studies, though, where use tests have been performed with single allergens, e.g. Johansen et al., who performed use tests with fragrances (6, 7). We recently employed the ROAT in guinea pigs, using cobalt chloride and colophony (8, 9).

Patch test reactions are generally evaluated by inspection and palpation. The use of objective methods, e.g. laser Doppler flowmetry (LDF), to quantify skin blood flow and measurement of transepidermal water loss (TEWL) to assess barrier function might enhance the accuracy of test reading and detect early changes. Such objective methods have been used on patch test reactions (10, 11) but, as far as I know, not on ROAT reactions, where test preparations are applied without occlusion.

The present aims were to investigate the outcome of ROAT and its relation to patch test results, in patients allergic to colophony and in colophony-negative controls, using colophony at different concentrations, and to follow ROAT sites visually and with two objective techniques.

MATERIALS AND METHODS

Subjects
Thirteen individuals (10 women and 3 men) with contact allergy to colophony and 9 controls (7 women and 2 men) with no history of colophony sensitivity participated in the study. The colophony-sensitive subjects were chosen among individuals patch-tested at our clinic during the past 6 years. Their mean age was 46 years (18–66 years) (Table I). The controls were healthy volunteers, with a mean age of 40 years (22–63 years). Criteria for entering the study were (a) a maximum of two positive test reactions to other substances of the standard series, apart from colophony (controls were not allowed to have a history of multiple contact allergy); (b) no personal history of atopic dermatitis; and (c) no dermatitis of the volar aspect of the forearms for at least a year prior to the study.

The colophony-sensitive subjects had been positive at the serial dilution patch test initiating the study, and the controls negative to colophony in the corresponding patch testing.

Materials
Portuguese colophony (Socer, Lisbon, Portugal) was used for patch testing in colophony-sensitive individuals and for ROATs. Controls were patch-tested with a standard colophony preparation (20% pet.) (Chemotechnique, Malmö, Sweden).

Vehicle for colophony. A vehicle of acetone and arachis oil 1:1 (w/w) (Apoteksbolaget, Stockholm, Sweden) was developed. Arachis oil was chosen since we are experienced with it, from use in animal testing at our laboratory. Different mixtures with acetone were tested in clinical trials. In humans the proportion 1:1 (w/w) seemed preferable, while in guinea pigs the proportion acetone/arachis oil 5:1 (w/w) was easier to apply (9).

Concentrations for serial dilution patch testing. The concentrations of colophony used were 20%, 10%, 1%, 0.1% and 0.01%. These concentrations were chosen in accordance with previous studies (12). Concentrations used for the ROATs. For the ROATs 20%, 1% and 0.1% concentrations were used. They were chosen since 20% is the standard concentration for patch testing and 1% is the limit for classification and labelling of allergic substances according to the Council Directive 88/379/EEC. 0.1% was the lowest concentration to cause a positive patch test reaction in the pilot study (see below). The colophony solutions were prepared daily at our laboratory and stored in the refrigerator when not in use.

“Positive” control–sodium lauryl sulfate. A 25% (w/w) water solution of SLS (sodium dodecyl sulfate, 99% purity, Fluka Chemie AG, Buchs, Switzerland) was chosen as “positive” control for the ROATs (13). In most previous studies, SLS was applied using patch test techniques, and no data was found on an optimal concentration for open application. Due to limitations in solubility, 25% was found to be the highest possible concentration.
### Table I. Results of serial dilution patch test with colophony in acetone/arachis oil 1:1 (w/w) (test concentrations: 20, 10, 1, 0.1, 0.01%) and repeated open application tests (ROATs) with colophony (20%, 1% and 0.1%) in 13 colophony-sensitive subjects

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Patch-test reaction to colophony 20%</th>
<th>Threshold for a pos. patch test reaction</th>
<th>First day* for a pos. ROAT reaction to</th>
<th>First day* for ≥100% rise at LDF to</th>
<th>First day* for ≥50% rise in TEWL? to</th>
</tr>
</thead>
<tbody>
<tr>
<td>no.</td>
<td>sex/age</td>
<td>score</td>
<td>conc.%</td>
<td>score</td>
<td>20%</td>
</tr>
<tr>
<td>1</td>
<td>F/47</td>
<td>++</td>
<td>10</td>
<td>+ +</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>F/61</td>
<td>+</td>
<td>100*</td>
<td>+</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>F/25</td>
<td>+ +</td>
<td>1</td>
<td>+</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>F/66</td>
<td>+</td>
<td>100*</td>
<td>+</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>F/53</td>
<td></td>
<td>10</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>M/48</td>
<td>+</td>
<td>1</td>
<td>+</td>
<td>3</td>
</tr>
<tr>
<td>7</td>
<td>F/52</td>
<td>+ +</td>
<td>10</td>
<td>+ + +</td>
<td>7</td>
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<tr>
<td>8</td>
<td>M/31</td>
<td>+ + +</td>
<td>1</td>
<td>+ +</td>
<td>2</td>
</tr>
<tr>
<td>9</td>
<td>F/47</td>
<td>+ + +</td>
<td>0.1</td>
<td>+</td>
<td>3</td>
</tr>
<tr>
<td>10</td>
<td>M/32</td>
<td>+ +</td>
<td>0.1</td>
<td>+ +</td>
<td>2</td>
</tr>
<tr>
<td>11</td>
<td>F/18</td>
<td>+</td>
<td>10</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>F/66</td>
<td>+ +</td>
<td>1</td>
<td>+ +</td>
<td>2</td>
</tr>
<tr>
<td>13a</td>
<td>F/38</td>
<td>+ + +</td>
<td>0.1</td>
<td>+ + +</td>
<td>1</td>
</tr>
</tbody>
</table>

* Day 0 = day of first examination and first application of test substances (after examination).

b Lasting for more than 1 day.  
1 Low value day 0. From Day 1 stable values over time.  
5 Patch test-positive also to 0.1%, but negative to 1%.  
3 Suntanned back.  
ROAT-positive on Day 4. Reactions had disappeared on Day 7 but reappeared on Day 11.  
4 Dry skin on arms, fluctuating values at several test sites, temporary rise.  
2 Application of 20% colophony stopped after 1 application and of 1% after 2 applications. No measurements of test sites treated with colophony 20% and 1% performed during second week.  
3 ROAT-positive on Day 4. Reactions had disappeared on Day 7.

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### Patch testing

Fifteen microlitres of each of the five colophony solutions, and the vehicle and the SLS solution (Table II) were applied in random order to the skin and spread over the area with a glass rod. The sites were left to dry for approx. 10 min before sleeves were allowed to be rolled down. Open applications were performed by an assistant, blind to the investigator. The applications were performed once daily – Monday to Friday the first week and Monday to Thursday the next week – at roughly 24-h intervals. Immediately prior to application, the skin of each marked area was examined. Examination was performed also on the second Friday. The subjects were allowed to take showers, but no baths, during the experimental period, and they were instructed not to use soap or moisturisers on the arms.

### Examination of test sites

**Visual assessment.** Each marked area was assessed visually and palpated, and all changes were noted and described, since there is no generally accepted reading scale for ROATs (15). It was classified as positive when there were five or more red macules or papules within

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### Table II. Number of test sites, in 13 patients allergic to colophony (Table I) and 9 controls, showing a positive ROAT reaction

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Subjects</th>
<th>Positive ROAT reaction on Day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>n</td>
</tr>
<tr>
<td>20% colophony</td>
<td>Patients 13*</td>
<td>6</td>
</tr>
<tr>
<td>1% colophony</td>
<td>Patients 13</td>
<td>1</td>
</tr>
<tr>
<td>0.1% colophony</td>
<td>Patients 13</td>
<td>-</td>
</tr>
<tr>
<td>25% SLS</td>
<td>Patients 13*</td>
<td>-</td>
</tr>
<tr>
<td>25% SLS</td>
<td>Controls</td>
<td>9</td>
</tr>
</tbody>
</table>

* Application of colophony stopped in 5 patients; last application: Day 0, Day 3 (3 patients) and Day 4.  
5 Treatments stopped in one patient; last treatment Day 8.  
4 Application stopped in 2 controls; last application on Days 3 and 4.

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*Acta Derm Venereol (Stockh) 78*
the marked test area. A ROAT reaction to SLS was considered positive when there was a confluent erythema.

**Laser Doppler flowmetry.** Blood flow was measured according to guidelines of the Standardization Group of the European Society of Contact Dermatitis (ESCD) (16). The blood flow at each premarked area of the arms was examined. The apparatus (Periflux, Perimed, PF1d, Stockholm, Sweden) and the measuring technique have been described elsewhere (17). The integrating probe with a probeholder PF107 was used (18). There was no contact between the skin surface and the probe. Blood flow – in arbitrary units – was recorded on a pen recorder (SE 120, BBC, Goerz Metrasatt, Vienna, Austria) for 2–3 min, depending on when stability was reached. An increase in blood flow units of ≥ 100% compared to baseline (pre-treatment, Day 0) was regarded as meaningful.

**Transcutaneous water loss (TEWL).** TEWL from test sites was measured using an evaporimeter, EPI, (Servo Med. Stockholm, Sweden) according to the ESCD guidelines (19). Room temperature varied between 19.6 and 22.4°C. Wearing an insulating glove, the operator held the probe in her hand and the measuring was performed inside a draught-shielding box. Data was recorded for 60 s using the Evaporimeter applications software package (EVM) on a personal computer (Digital). The value (g/m² h) recorded is the mean value of the last 15 s. The value presented is the mean of two recordings. An increase in TEWL of ≥50% compared to baseline (pre-treatment, Day 0) was regarded as meaningful.

**Pilot study.**

The vehicle developed was tested in healthy controls prior to the study and no visible reactions were observed. Five individuals, outside the main study but with known contact allergy to colophony, participated in a pilot study including patch testing with a serial dilution of colophony in petrolatum in parallel with such a dilution in the vehicle developed: acetone/arachis oil 1:1 (w/w). The patch testing was followed by daily open applications of colophony in arachis oil/acetone, according to the same routines as in the main study. The tests were not, however, read blindly and the reactions were followed only visually. The results of the pilot study are presented in Table III.

The main study took place from September 1996 to January 1997. It was approved by the Karolinska Hospital Ethical Committee.

### RESULTS

**Patch test results.**

The results of the patch testing in the 13 colophony-sensitive subjects are shown in Table I. The lowest consecutive test concentration, at the serial dilution patch test with colophony, to which the subject had at least a + reaction, is defined as the threshold concentration. There were no reactions to the vehicle.

**Outcome of ROATs with colophony – visual assessment.**

The results are presented in Tables I and II. Ten of the 13 colophony-allergic subjects had a positive ROAT reaction to the 20% colophony solution and 4 of these also reacted to 1%.

The threshold concentrations at patch testing in relation to the ROATs outcome are presented in Table IV. Among the controls there were no reactions to the vehicle and there were no positive ROAT reactions to colophony (Table I).

Reactivity at test sites started as scattered, small red macules or papules, often spreading outside the marked area. With continued applications the macules and papules became more numerous, and in 5 subjects (nos. 4, 8, 10, 12 and 13, Table I) the reaction to 20% colophony eventually turned into manifest eczema and the application of colophony was stopped. One subject (no. 13, Table I) developed itchy eczema, spreading outside the test area after one application of the 20% colophony and two applications of the 1% colophony solutions.

**Outcome of ROATs with SLS – visual assessment.**

Five of 13 colophony-sensitive subjects and 7/9 controls developed a visible reaction to 25% SLS. The first day of appearance varied from Day 2 to Day 11 (Table II). The SLS reaction started as a weak redness covering the whole treated area, but not outside. Later the redness deepened and the skin became dry.

**Laser Doppler flowmetry.**

The median blood flow values for the non-treated and vehicle-treated sites were stable throughout the experimental period. At least a 100% rise of the value at the test sites treated with 20% colophony was seen in all subjects but one, who had a positive ROAT reaction (Table I). Generally the rise (≥100%) was noticed on the same day, or after the reaction was considered positive visually. In most cases the increase stopped with the discontinuation of treatment, and the value was lower.

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### Table III. Results from the pilot study: serial dilution patch test with colophony in two different vehicles and repeated open application test (ROAT) assessed visually in 5 subjects with contact allergy to colophony

<table>
<thead>
<tr>
<th>Subject</th>
<th>Threshold for a positive patch test reaction to colophony</th>
<th>First day* for a positive ROAT at different concentrations of colophony</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>sex/age in pet. in acetone/arachis oil (1:1)</td>
<td>conc. % score</td>
</tr>
<tr>
<td>A</td>
<td>F/45</td>
<td>10b</td>
</tr>
<tr>
<td>B</td>
<td>F/49</td>
<td>0.1</td>
</tr>
<tr>
<td>C</td>
<td>M/45</td>
<td>1</td>
</tr>
<tr>
<td>D</td>
<td>F/29</td>
<td>0.1</td>
</tr>
<tr>
<td>E</td>
<td>M/38</td>
<td>1</td>
</tr>
</tbody>
</table>

* Day 0, Day 1 – see note in Table I. b Doubtful reaction also to 1%. c Doubtful reactions also to 0.01 and 0.001%.

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*Scattered papules around test area on Day 4, reactions had disappeared on Day 7 and did not reappear, despite further applications.*
Repeated open application tests in patients allergic to colophony

on Day 7, after the weekend; but there were exceptions. Blood flow values for 4 subjects (nos. 2, 3, 8 and 12, Table I) are presented in Fig. 1. Only in three test sites treated with 1% colophony was an increase in skin blood flow units of ≥100% seen (Table I).

**Clinical observations**

One colophony-sensitive subject had hand eczema and one mild eyelid eczema, on entering the study. Apart from eczema on or around test sites, none developed dermatitis during the observation period. The subjects reported very little discomfort from the test areas.

**TEWL measurements**

The median values and the 25% and 75% percentiles of TEWL for the 10 measurements (Day 0–Day 11) of the non-treated sites in all 22 participants (13 colophony-sensitive subjects and 9 controls) are presented in Fig. 2. In 5/10 subjects with a positive ROAT reaction to 20% colophony there was, at that test site, a ≥50% increase in TEWL lasting for more than a day. In all cases the rise came after the visual reaction, in 4 not until the second week of treatment and in some after the application had stopped (Tables I and II).

Most subjects, some with no visually positive ROAT reaction, showed an increase in TEWL with the application of 25% SLS. In some individuals the rise came before the visible reaction.

**DISCUSSION**

**Outcome and concordance of patch tests and ROATs**

The present study shows a correlation between the results of serial dilution patch testing and the outcome of ROATs in humans tested with colophony (Table IV). With a high threshold concentration (10%) half the subjects had a positive ROAT reaction to the 20% colophony preparation; with a lower threshold concentration (1% or 0.1%) all reacted in the ROAT (p = 0.07, in Fisher’s exact test, Epi Info). The only subject with a 10% threshold concentration to react in ROAT with 1% colophony was a woman with a suntanned back, which might have influenced the result of the patch testing (no. 4, Table I). Other studies have also shown concordance between thresholds of sensitivity in patch testing and the outcome of use tests, e.g. for Kathon CG (20), cinnamic aldehyde (6) and isoeugenol (7). In a recent experimental study in guinea pigs (9), we found concordance between patch test results and outcome of ROATs and a dose-response relationship in ROAT for cobalt chloride as well as for colophony.

In the study with cinnamic aldehyde (6) a relationship was found between the strength of patch test reactions, when a standard concentration had been applied, and the outcome of ROAT. Another study showed a correlation between scores at sites tested with 1,000 ppm formaldehyde and the results of serial dilution patch testing (15). Such tendencies were seen also in this study (Table I), but there was no strong relationship between the scores of the patch test reactions to 20% colophony and the ROAT outcome.

Hannuksela & Salo suggest in their original paper on ROAT (5) that test substances should be applied twice daily for 7 days. In the present study we cannot predict whether more subjects would have reacted in the ROATs to lower concentrations of colophony or more quickly if applications had been performed twice daily, or during the weekend as well as on weekdays. It is notable, though, that 9 of the colophony-sensitive subjects reacted in the ROATs within the first 5 days.

**Table IV. Outcome of ROATs with colophony in relation to threshold concentration of colophony at patch testing in 13 colophony-sensitive subjects**

<table>
<thead>
<tr>
<th>Threshold concentration of colophony for a positive patch test reaction</th>
<th>Outcome of ROATs with colophony. Use test conc. and no. of subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetone/arachis oil 1:1 (w/w) was used as vehicle.</td>
<td>20%</td>
</tr>
<tr>
<td>Use test conc.</td>
<td>Pos</td>
</tr>
<tr>
<td>10% (n=6)</td>
<td>3</td>
</tr>
<tr>
<td>1% (n=4)</td>
<td>4</td>
</tr>
<tr>
<td>0.1% (n=3)</td>
<td>3</td>
</tr>
</tbody>
</table>

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(Tables I and II). Test preparations were applied only once a day, so that the applications could be made by a skilled assistant and not by the subjects themselves, in order to maintain a standard amount of test substance and mode of application. With five different test preparations, the risk of error would also have been great if the subjects had applied them themselves. The weekend break was accepted for practical reasons and to favour compliance. It would have been interesting if further use test concentrations could have been applied. The number of possible test sites on the arms was, however, a limiting factor. Regarding the clinical relevance of the test concentrations chosen, we know that soldering fluxes for electronic assemblies and some glues contain 20% colophony. Products containing modified colophony also contain unmodified colophony in amounts from 15% (Abitol®, 21) and below. Thus a test concentration of 0.1% could correspond to colophony remaining in highly modified products. The present limit for classification and labelling of chemical products containing colophony – 1% – gave a positive ROAT reaction in 4 subjects, and 2 subjects (Table I, Table III) showed some reactivity also to 0.1%. No “safe” concentration of colophony could be established from this study.

The ROAT reactions to colophony differed morphologically from patch test reactions, as is also described for ROATs with cinnamic aldehyde (6). In a study with formaldehyde (15) the ROATs were regarded as negative, although a few papules were seen at some test sites. In the present study, where ROAT reactions were followed day by day, even minimal reactions at the test site – e.g. ≥ 5 red macules or papules – were regarded as positive, since they intensified during the observation period. In half the subjects with a positive ROAT, the reaction never turned into eczema despite further applications of colophony, while 2 subjects (no. 13, Table I, and D, Table III) showed widespread reactions after only one application of a small amount of colophony. None of these 2 subjects had any ongoing eczema elsewhere. Parallel patch testing in the pilot study shows that the choice of vehicle influences the test results (Table III). Petrolatum was not suitable for ROAT due to extensive spreading outside the test area, which was also seen in guinea pigs (8).

Bioengineering techniques when evaluating ROATs

Although there were some intra- and interindividual differences in the values over time, both TEWL and blood flow values for the whole group, measured at the non-treated and the vehicle-treated sites, were stable (Fig. 2), indicating good measuring conditions. It was impossible to decide for each test site when there was a statistically significant increase in blood flow units or TEWL. As a limit for presentation, a ≥ 100% and ≥ 50% increase, respectively, was chosen, since in most untreated and vehicle-treated sites the variation over time did not exceed these limits. The blood flow values varied considerably between different subjects (Fig. 1). In patch testing with irritants, skin blood flow must be increased 3–4 times before the naked eye can detect an erythema (11, 22), while a positive reaction at the ROAT sites, treated with colophony, was seen before any significant increase in blood flow was recorded. With the morphology noted – scattered erythema – this is, however, what might be expected. One of the goals – to detect early changes and to quantify them – was thus not achieved.

SLS-testing

SLS was used as a positive control. The highest concentration possible (25%) was therefore used. However, there was great variability in the reactivity to SLS, both visually and with regard to barrier function, with several subjects not reacting. This was also found by Lammintausta et al. when performing ROATs with SLS 2%, 5% and 7.5% (23). In most previous studies (13, 24) where bioengineering techniques were used, SLS was applied under occlusion. More controls than colophony-sensitive subjects reacted to 25% SLS. I have no other explanation for this finding but chance.

Number of participants and participation

A greater number of participants would have been desirable. This study was time-consuming for the subjects though, and with the criteria for inclusion (no atopic dermatitis or multiple contact allergy) the number of possible participants was limited. Four individuals were excluded because of a negative serial dilution patch test. Avoidance of atopic dermatitis seemed important, since atopic skin might influence the TEWL (25).

All participants completed the whole study, except for TEWL and LDF measurement in one allergic subject (no. 11, Table I) and one control, on Day 7 and Day 9, respectively. The test sites were, however, inspected and the test substances were applied. Studies like this, lasting for some time and involving humans, naturally admit several sources of error. To minimize them the application of test substances was standardized and all test substances, both for patch testing and for ROATs, were applied in random order, blind to the examiner, and with a few exceptions the author performed all daily test readings, LDF and TEWL measurements.

Conclusions

An ordinary patch test, performed with a standard concentration of colophony only, is insufficient to give information on reactivity to open colophony exposure in colophony-sensitive subjects. There is a relationship between the results of a serial dilution patch test and the outcome of ROATs. A use test, such as the ROAT, or a serial dilution test, might therefore be useful in assessing the clinical relevance of contact allergy to colophony. There is great interindividual variability in reactivity to open applications of colophony in colophony-sensitive individuals. The vehicle used might influence the outcome of patch test reactions and probably also of ROATs.

The use of two bioengineering methods (TEWL, LDF) seemed to add no further information when evaluating the test sites in ROATs performed with colophony.

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