Abstract

Two newly developed tests for gustatory sweating, providing both quantitative and topographic information, are presented. In both tests a paper stencil shaped to fit the complex anatomy of the parotid region is used. The blotting paper technique uses the difference in weight before and after gustatory stimulation to measure the amount of sweating. The iodine-sublimated paper histogram (ISPH) uses iodine sublimated office paper that changes colour when wet. The paper stencil is then digitized and a histogram algorithm applied to measure the area of sweating. A calibration of these tests with known and appropriate quantities of saline is presented.

Reference


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New Objective and Quantitative Tests for Gustatory Sweating

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Two newly developed tests for gustatory sweating, providing both quantitative and topographic information, are presented. In both tests a paper stencil shaped to fit the complex anatomy of the parotid region is used. The blotting paper technique uses the difference in weight before and after gustatory stimulation to measure the amount of sweating. The iodine-sublimated paper histogram (ISPH) uses iodine sublimated office paper that changes colour when wet. The paper technique uses the difference in weight before and after gustatory stimulation to measure the amount of sweating. The presented. In both tests a paper stencil shaped to fit the complex anatomy of the parotid region is used. The blotting paper technique uses the difference in weight before and after gustatory stimulation to measure the amount of sweating. The iodine-sublimated paper histogram (ISPH) uses iodine sublimated office paper that changes colour when wet. The paper stencil is than digitized and a histogram algorithm applied to measure the area of sweating. A calibration of these tests with known and appropriate quantities of saline is presented. Key words: facial nerve, Frey syndrome, gustatory sweating, histogram, parotidectomy, sweat glands, test, topographic.

In 1923, Lucie Frey described the “syndrome du nerf auriculotemporal” (1), characterized by lateral facial sweating and flushing during meals. Testing for Frey syndrome has in general been limited to sweating, since this is the most troublesome symptom. Only Laage-Hellman investigated flushing, and this was done by simple direct observation and without any quantification (2, 3). Frey syndrome testing can therefore be considered to represent the assessment of the function of facial eccrine sweat glands.

The major function of the eccrine sweat glands in humans is thermoregulation (4, 5). About 2–4 million sweat glands are distributed over the entire human body with a density varying from 60 glands/cm² on the back to 600 glands/cm² on the palms and soles (6). The maximal sweat rate is about 2–20 nl/min/gland (4). The face has about 250 glands/cm² (7) and thus the maximal facial sweat rate should be about 0.5–5.0 µl/min/cm².

Tests of sweat output function measure the production of sweat by a group of sweat glands and these test could be classified as: i) “topographic”, where a chemical reaction provides a view of the anatomical location of sweat secretion; ii) “electric”, where a change of skin impedance by the humidity of sweat is measured; and iii) “thermodynamic”, where the skin humidity is evaporated and a “sudometer” measures the thermal mass of the air stream (8). Advantages of the electric and thermodynamic tests include the possibility of repeated and dynamic measures, while topographic tests give a better representation of surface and anatomical distribution.

A useful test method for Frey syndrome should provide topographic information and possibly quantification of the amount of sweating. While dynamic measures might be interesting in investigation studies, their use in clinical practice is of limited value. Further characteristics of the test should include: i) simplicity of the method; ii) sensitivity; iii) reliability; iv) adequate dynamic range, so that different sweating rates could be appreciated; v) absence of toxicity and allergenicity of the agents used; vi) easy removal from the skin of the applied agents; and vii) low cost (6).

The most frequently used method for sweat secretion assessment for Frey syndrome was originally described by Victor Minor, a Russian neurologist (9). A solution containing 1.5 g iodine, 10 g castor oil and 88.5 g of absolute alcohol is painted on the skin. After drying, the areas are powdered with starch. The water in the sweat produces blue colouring of the iodine-starch mixture. With limited sweat production, the apertures of individual sweat glands are marked as small blue dots, while with larger amounts of sweat secretion the blue dots enlarge and eventually become confluent. The Minor test is a topographic method allowing accurate mapping of the involved surface. Photographs can be taken. Disadvantages include the necessary application of several layers of regents, the difficulty of removing the iodine paint, possible allergy to iodine, the difficulty of using the method with heavy perspiration and the lack of dynamic testing (10). The Minor test is cumbersome and, not infrequently, patients refuse it (11).

Modifications of Minor’s test include the use of other dyes, such as bromophenol blue powder (12), pyrogallol (13) and quinazarin (14). Each of these methods shares the above disadvantages of the Minor test.

Since the available methods appear cumbersome and qualitative at best, two new techniques were developed that provide an objective, quantitative and topographic measurement of sweating. Prior to their use in Frey syndrome patients, we would like to report on their standardization and calibration.
METHODS

In order to assess the sweating of a complicated topographic surface, such as the lateral face, including the pre- and post-auricular areas, a custom stencil was designed (Fig. 1). In both methods, the stencil is to be applied on both sides of the face during a gustatory stimulation and gently pressed by the investigator with gloved hands onto the patient’s skin, for a duration of 1 min.

**Blotting paper technique**

The blotting paper technique is a quantitative measure of the amount of sweat. A paper stencil is cut from commercial blotting paper, weighted, applied to the face during a gustatory stimulation and weighted again. The weight change is taken to represent the amount of sweat absorbed.

For the calibration, known quantities of 0.9% solution of sodium chloride were applied on the stencils. The following amounts were used 2, 5, 10, 25, 50, 100 and 250 μl, delivered by commercial micropipettes. For each amount the measure was repeated three times and the results reported as an average ± SD.

We initially noticed wide and erratic variations when weighting these stencils. The source of error was pinpointed to the distribution of the weight over the surface of usual commercial balances, and possible movements of the folded stencils. To correct this, the folding of the stencils was standardized and a cut made to stabilize the folded paper and prevent its movements. With this technique, the margin of error (test-retest variation) of the method is around 2 mg (2 μl).

**Iodine-sublimated paper histogram (ISPH)**

The ISPH method that we propose can be seen as a modification of the classical Minor test. ISPH provides a quantitative evaluation of the amount of sweat produced and a topographic image of the sweat-producing area.

The ISPH method uses regular office paper sheets, which have been sublimated with iodine. One hundred office paper sheets are placed in a glass jar and exposed during 2 weeks to iodine vapour (1 g Iode resublimiert I-3380, Sigma AG, Deisenhofen, Germany). The iodine sublimated paper takes on an amber colour. Wetting this paper results in a localized colour change from amber to blue, similarly to colour changes of the Minor iodine-starch mixture. As with the Minor test, this colour change corresponds to the reduction of starch by water.

The iodine-sublimated paper sheets were cut according to the predefined stencil, and applied to the face during a gustatory stimulation. After this, the stencil was scanned, and the digital image was subjected to a histogram algorithm (Fig. 2). We used a Scanjet II (Hewlett Packard) scanner in an 8-bit grey scale mode with a resolution of 70 dpi (28 points/cm or 784 points/cm²). The histogram calculation was performed with the algorithm of the image analysis module of Mathlab (The Math Works Inc., Natick, MA, USA). The clear tone of the stencil background was used as a threshold value. The histogram data were divided in 3 equal bins of increasing darkness. The darkest bin corresponds to paper zones in which the starch is supposed to be totally reduced by the applied water (sweat). Only the surface of this darkest bin was taken into account to compute the wet surface.

The technique was calibrated by applying known quantities of 0.9% solution of sodium chloride on the stencils. The following amounts were used 2, 5, 10,
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**RESULTS**

The results of the application of known quantity of a 0.9% NaCl solution on the stencils of blotting paper and those of the ISPH method are shown in Table I and Fig. 3.

Using the blotting paper technique, repeated measurements with the same stencil showed the precision of the measure to be 2 mg. This corresponds to 2 ml water. The amount of sweat collected with the blotting paper technique was close to the amount of saline, actually applied. One exception is 2 ml, an amount, which is near the resolution of the method.

The correlation between the amount measured with the blotting paper technique and the surface calculated with the ISPH is excellent (Pearson correlation coefficient = 0.998; \( p < 0.001 \)).

**DISCUSSION**

According to Laage-Hellman (2) and our personal data (unpublished observations), about 40–50% of patients after parotidectomy are aware of their gustatory sweating.

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Table I. Normative data for gustatory sweating

<table>
<thead>
<tr>
<th>Quantity of solution (µl)</th>
<th>Sweat quantity measured with the blotting paper technique (mg)</th>
<th>Sweat area measured with ISPH (cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average</td>
<td>SD</td>
</tr>
<tr>
<td>2</td>
<td>2.38</td>
<td>2.13</td>
</tr>
<tr>
<td>5</td>
<td>4.00</td>
<td>0.62</td>
</tr>
<tr>
<td>10</td>
<td>9.89</td>
<td>0.90</td>
</tr>
<tr>
<td>25</td>
<td>24.98</td>
<td>0.53</td>
</tr>
<tr>
<td>50</td>
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<td>0.56</td>
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</tr>
<tr>
<td>250</td>
<td>244.04</td>
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</tbody>
</table>
tory symptoms and 80–90% of patients exhibit a positive objective test. While earlier prevention techniques have been ineffective or were associated with serious potential side-effects, interest in using different materials as subcutaneous barriers at the end of parotidectomy has been renewed recently (15, 16). In addition, the treatment of established Frey syndrome with botulinum toxin intradermal injection has recently been advocated (17–19). Both of these modalities should be evaluated with an objective and quantitative test. Furthermore, mapping of the skin area to be injected with botulinum toxin is paramount to the success of this technique.

The only previously accepted test for sweating has been the Minor test. While it can be seen as objective, it is not a quantitative test and therefore might not be suited for the comparison of two treatments. The topographic information provided by the Minor test is cumbersome to use with local injection techniques such as the infiltration of botulinum toxin. The skin area should be cleaned from the reagents applied and disinfected prior to injection, while maintaining a mapping of the topographic information. Finally, this injection treatment is proposed to the patients with the most troublesome gustatory sweating in whom the sweat tends to drip down the facial and cervical skin. In these patients mapping with the Minor test is quite imprecise because an intense blue smear covers involved skin and the dripping blue-coloured sweat obscures the assessment of the more dependently located skin.

The tests described satisfy most of the requirements of an ideal sweat test, as defined by Sato et al. (6): quantitative test, simplicity, sensitivity, reliability, adequate dynamic range, absence of toxicity and allergenicity, easy removal from the skin of the applied agents and low cost. The blotting paper technique recorded weight changes close to the amount of saline actually applied (Table I). With increasing amounts of saline applied, the blotting paper technique measured higher weights and the surface of the ISPH method was larger. More importantly, an excellent correlation was obtained between the results of both methods. In addition, these two tests give complementary data. While the quantitative data obtained with the blotting paper method is excellent, the topographic information is sub-optimal. However, the topographic data of ISPH technique is excellent, providing a mirror image on the facial sweating area (19). Probably the only disadvantage of the ISPH method in testing Frey syndrome is the lack of dynamic results. While dynamic measures might be interesting in investigation studies, their use in clinical practice and more specifically in gustatory sweating appears of limited value. Laccourreye et al. recently proposed the use of a L-lactate skin electrode for the measurement of sweat gland output (20). This technique appears interesting since lactate concentration in sweat is 10–20 times that of plasma (4, 5), no signal is detected in normal skin and dynamic results can be obtained. Serious disadvantages for its application in Frey syndrome are that no topographic data are generated and that it uses expensive and complicated equipment.

The excellent correlation of these two methods can be used as an internal validation, if one wishes to use only one of these methods. The blotting paper has the advantages of being simple and of actually measuring the amount of sweat produced; however, no topographic data are obtained. The ISPH method is slightly more complicated, but, in our opinion, still much simpler than the traditional Minor test. Depending on the goals pursued, the digitalization aspect can be omitted, while still obtaining excellent topographic data.

Also, our initial results in patients show these two evaluation techniques to be extremely well tolerated. We have used these techniques in 70 patients, in which the results of different subcutaneous barriers were placed for the prevention of Frey syndrome (unpublished observations), as well as 16 patients treated with botulinum toxin (unpublished observations). Because these tests are well tolerated bilateral information is easily obtained and can be used as internal control. Probably the most useful application is in testing prior to botulinum toxin injection, where precise topographic information is obtained without the application of any product to the face. After the test, the skin is wiped with a regular alcoholic swap and the injection can begin immediately. If necessary the ISPH stencil can be brought back close to the facial skin in order to obtain further precision in the exact skin area involved.

REFERENCES

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