Measurement of Sebum Output Using a Lipid Absorbent Tape

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A sebum absorbent tape is introduced as a reproducible and convenient method for estimation of sebaceous gland output. We have tested the reproducibility of this method by serial measurements of sebum excretion rates (SER) of 10 individuals over a 6-week period, and in addition we have correlated this method with the conventional hexane extraction technique. The sebum absorbent tapes gave consistent values for the SERs, and within subjects variation over the 6-week period was statistically nonsignificant. A coefficient of variation for the tapers was calculated as 16.25 ± 6.78% based on these serial measurements. Furthermore, the amount of total lipid collected using this technique \( (n = 16) \) correlated well with the hexane extraction technique, \( r = 0.89 \). Free fatty acids \( (r = 0.87) \), triglycerides \( (r = 0.92) \), wax and cholesterol esters \( (r = 0.83) \), and squalene \( (r = 0.88) \) also showed a good correlation. Cholesterol occasionally suffered from incomplete separation on thin-layer chromatograms; however, a sample cleanup procedure was developed for tape extracts that removed interfering materials and allowed complete separation of all sebum components. J Invest Dermatol 87:260-263, 1986

**M**onitoring sebaceous gland activity in humans has been achieved by a variety of techniques in which the amount of sebum that reaches the surface of the skin during a specific period of time is determined. This measurement is referred to as the sebum excretion rate (SER) and is an indirect measure of the amount of sebum produced. The first technique developed for measuring SER involved gravimetric analysis of sebum absorbed onto cigarette papers [1] and has served as the backbone of numerous investigations. Subsequently other techniques such as the extraction of skin surface lipid followed by quantitation with thin-layer chromatography (TLC) with reference to known standards [2], the lipometre [3], and ground glass photometric techniques [4,5] have been described. Most recently, a technique based on the collection of lipids onto bentonite clay has provided a very accurate method for collecting lipids and measuring SERs [6].

All of these techniques have their merits, but also have some limitations. The cigarette absorbent paper technique requires careful attention to ensure adherence to the skin surface and absorbency of the papers may vary from batch to batch [7]; solvent extraction, on the other hand, is subject to sample loss, and the ground glass techniques are not accurate in extreme ranges of sebum production [8]. The bentonite clay technique, although accurate, requires extensive preparation of the site and the total sampling period exceeds that of the other techniques. Recently, a sebum absorbent tape has been developed which adheres to the skin surface visualizing individual sebum droplets. In this communication, we describe the use of this tape for measuring sebum excretion rates and for the quantitation of sebum components.

**MATERIALS AND METHODS**

The Sebu-Test Strip (CuDerm Corp., Dallas, Texas) is comprised of an open-celled, microporous, hydrophobic polymeric film that is coated with an adhesive layer that will permit the passage of lipids as the strip tightly adheres to the skin surface (Fig 1). **Reproducibility** The reproducibility of the tape was evaluated by serially measuring the SER on the left and the right sides of the foreheads of the same panel of 10 adult males at weekly intervals for a period of 6 weeks. Samples were collected between 9 AM and 12 noon. Prior to application of the adhesive strip cut to 4.7 cm² onto the face, the skin was cleaned of debris by washing with soap and water, then defatted by wiping with a gauze pad saturated with hexane. Once the skin was dry, a Sebu-Test Strip was peeled from its backing paper using defatted forceps and affixed to the cleaned surface with gentle pressure to assure adequate adhesion. Surgical gloves were worn by the person handling the tape. The subject was then asked to remain at rest for 3 h. At the completion of the lipid collection, the Sebu-Test Strips were removed and placed in acid-washed, Teflon-capped screw-cap vials.

**Extraction and Quantitation of Lipid** In the laboratory 2 ml of hexane, containing an internal standard of 80 μg methyl neronate, was added to the vials containing the tapes. The internal standard was incorporated to correct for spotting error during TLC. The tightly capped vials were sonicated for 5 min at room temperature in a Branson sonicator. After transferring the fluid contents of the vial to a second vial, another 2 ml of hexane were added and the vial was shaken vigorously. The resulting solvent sample was pooled with the first collection and dried under a stream of nitrogen at 40°C. The dried samples were stored under nitrogen at -20°C until processed for TLC according to the

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**Abbreviations:**
- PVP: polyvinylpyrrolidone
- SER: sebum excretion rate
- TLC: thin-layer chromatography(gram)
procedures of Ruggieri et al [2]. Briefly, this entailed redissolving the thawed lipid samples in 0.2 ml of hexane and applying 5-μl aliquots onto 250 μm-thick 20 cm × 20 cm silica gel G TLC plates (Analttech Inc., Newark, Delaware). The plates had been precleaned in chloroform:methanol 2:1 and activated at 130°C for 60 min. Commercial reference standards of cholesterol, cholesterol oleate, oleic acid, methyl oleate, triolein, cetyl oleate, and squalene (Nu Chek Prep Inc., Elysian, Minnesota and Sigma Chemical Co., St. Louis, Missouri) were made up as a standard reference mixture (1 μg/5 μl each) in hexane and the mixture was applied as 5 μl onto the plates. After sample application the plates were developed according to the solvent system of Downing [9]. At the end of development the plates were allowed to dry, and the lipids were then visualized by spraying with 75% H2SO4 and heating to 220°C for 55 min. The charred chromatograms were quantitated using a Schoeffel model SD3000 dual-beam photodensitometer interfaced to a Spectra-Physics SP4100 computing integrator.

Comparison of the Sebum Absorbent Tape with the Hexane Extraction Technique The sebum absorbent tape was also compared with the hexane extraction technique of Ruggieri et al [2]. The left side of the foreheads of 16 adult males were sampled using the absorbent tape and concurrently the right side of the foreheads of the same panel were sampled using the hexane extraction technique. The latter entailed cleaning the skin surface by wiping with a gauze pad, moistened with a 1.0% solution of the nonionic detergent Triton X-100. The sampling site was then defatted using another gauze pad saturated with hexane. After allowing the surface to dry, the site was protected by a plastic weighing boat taped onto the skin by its edges. The roof of this chamber was perforated to allow evaporation of sweat and prevent a rise in skin surface temperature. After a 3-h period, this protective covering was removed and the lipids that had reached the surface were collected by pipetting 2 ml of hexane with 80 μg of methyl vernonate into a glass cup which covered a 3.8-cm² area of the skin. A Teflon rod was then used to agitate the surface of the skin for 30 s and the resulting mixture was transferred to an acid-washed, screw-capped vial. The collection was repeated with fresh hexane and the samples were pooled, dried under a stream of nitrogen at 40°C, and stored at −20°C until analysis by the TLC procedure outlined above.

Further Purification of Lipid Extracts Some adhesive component of the tapes was observed to tail into the region of the TLC plate where the cholesterol is found after routine development with the system of Downing [9]. This problem could largely be overcome by allowing the last solvent to migrate higher (to 15 cm) rather than the usual 10 cm distance. However, 2 additional methods for avoiding this potential source of interference were evaluated. In one method extracts of the tapes were also spotted onto 20 cm × 20 cm, 250 μm-thick silica gel G plates (Analtech Inc.) and developed in chloroform:ethyl acetate 94:6 (v/v) [10] in order to specifically resolve the cholesterol spot. The second approach utilized a minicolumn cleanup procedure to effectively remove the adhesive from the extracts prior to TLC.

Column Preparation A Sep-Pak silica gel minicolumn (Waters Associates, Milford, Massachusetts) was attached to a syringe barrel and the assembly was supported in an upright position. The syringe plunger was then used to wash the column with 5 ml acetone. The silica absorbent of the Sep-Pak was then modified with polyvinylpyrrolidone (PVP) (GAF Corporation, Wayne, New Jersey) so that it would act in a manner similar to TLC silica which contains PVP in the binder. A solution of PVP was prepared by dissolving 1.0 g of PVP completely in 20 ml ethanol; 80 ml of acetone was added and the solution was mixed. A 2-ml aliquot of the PVP solution was then pipetted into the column and slowly forced through. The plunger was then removed and the inside of the barrel and the column were washed twice with 1–2 ml of acetone. Similarly, the Sep-Pak was washed with 10 ml of diethyl ether:chloroform:hexane 5:3:2 (v/v/v). Prior to use this solvent system was saturated with 0.5 g sodium acetate per 10 ml solvent; the solvent was decanted from the excess sodium acetate. The Sep-Pak column was then used to elute the Sep-Pak column with diethyl ether:chloroform:hexane, which was then run through the Sep-Pak and slowly into the vial. The cleaned extract was then dried under a stream of nitrogen.

Recovery of Lipid Sample recovery after cleanup on the minicolumn was tested by preparing a standard mixture consisting of cholesterol, cholesterol oleate, oleic acid, methyl oleate, triolein, cetyl oleate, and squalene (at 1 μg/5 μl each in hexane) (Nu Chek Prep Inc. and Sigma Chemical Co.), including a sebum absorbent tape strip. This mixture was run through the Sep-Pak column in triplicate according to the above procedure. The amount recovered was compared with 3 aliquots of the same standard mixture that had not been run through the column. Quantitation was carried out by densitometric scanning of the charred chromatograms, as described above.

RESULTS

Reproducibility of the Sebum Absorbent Tapes The 3 h sebum excretion rates measured at weekly intervals over a period of 6 weeks gave highly reproducible values. Within subjects variation over this period was statistically nonsignificant, and the mean coefficient of variation was 16.25 ± 6.78% (Table I). Furthermore the values for SERs for the left and the right sides of the foreheads were well correlated: r = 0.94, week 1; r = 0.82, week 2; r = 0.89, week 3; r = 0.86, week 4; r = 0.91, week 5, and r = 0.75, week 6.

No statistically significant differences were found between the amount of total lipid (μg/3 h/cm²) or between each of the lipid components measured with the sebum absorbent tapes and the hexane extraction technique (Table II). The linear correlation of total lipid was r = 0.89 (Fig 2) and the following values were obtained for each individual component: free fatty acids, r = 0.87; triglycerides, r = 0.92; wax and cholesterol esters, r = 0.83; and squalene, r = 0.88. The correlation of cholesterol, was, however, rather poor, r = 0.29.
Table I. Within Subjects Variation in Sebum Excretion Rate over a 6-Week Period (µg/cm²/3 h)

<table>
<thead>
<tr>
<th>Subject</th>
<th>Right Side</th>
<th>Left Side</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>SD</td>
<td>%CV²</td>
</tr>
<tr>
<td>1</td>
<td>96.9</td>
<td>11.9</td>
</tr>
<tr>
<td>2</td>
<td>50.0</td>
<td>5.4</td>
</tr>
<tr>
<td>3</td>
<td>58.6</td>
<td>18.0</td>
</tr>
<tr>
<td>4</td>
<td>107.7</td>
<td>13.7</td>
</tr>
<tr>
<td>5</td>
<td>80.0</td>
<td>19.7</td>
</tr>
<tr>
<td>6</td>
<td>55.3</td>
<td>3.2</td>
</tr>
<tr>
<td>7</td>
<td>101.3</td>
<td>8.9</td>
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<td>8</td>
<td>96.5</td>
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<tr>
<td>9</td>
<td>89.0</td>
<td>11.8</td>
</tr>
<tr>
<td>10</td>
<td>116.9</td>
<td>12.2</td>
</tr>
</tbody>
</table>

Mean % CV (n = 20): 16.25 ± 6.78

²Coefficient of variation (% SD/mean).

Development in Chloroform: Ethyl Acetate A highly satisfactory separation of cholesterol from other components of skin surface lipid was achieved with the chloroform: ethyl acetate 94:6 (v/v) solvent system (Fig 3). Free fatty acids migrated close to the origin; triglycerides, wax, and cholesterol esters as well as squalene, close to the solvent front.

Sample Purification Sample purification using the Sep-Pak columns completely removed the adhesive and allowed a clean separation of all components on TLCs (Fig 4). Recovery of lipid was highly satisfactory; of the 1 µg of each component that was applied onto the column, the following quantities were recovered: cholesterol 0.96 ± 0.21 µg, free fatty acids 0.91 ± 0.12 µg, triglycerides 0.88 ± 0.07 µg, methyl oleate 0.95 ± 0.09 µg, wax esters 1.01 ± 0.05 µg, cholesterol esters 0.94 ± 0.04 µg, and squalene 0.94 ± 0.26 µg.

DISCUSSION

The sebum absorbent tapes provide a convenient and reproducible measurement of sebum excretion rates. We were able to demonstrate that the coefficient of variation, calculated from weekly measurements of sebum production was 16.25 ± 6.78%. Similar values have not been published for other methods for SER measurement; however, it can be estimated that the variation for the sebum absorbent papers according to the original description of the technique was of the order of 15.6% [1]. Cunliffe and Shuster [11] introduced slight modifications to the technique and the variation in this instance can be estimated to have been 21.2%. Similarly, the hexane extraction technique can be calculated to have a variance of 14.6% [2]. The SER of the individuals in the 6-week reproducibility study represented a range of 171.55 µg/cm² ± 82.47 µg/cm² and the tapes did correlate well with the hexane scrub technique at high (327.68 µg/cm²) and low (50.6 µg/cm²) SER. Furthermore, the tape has been extensively applied to monitoring sebum production in acne patients with high SERs, and the results have been highly satisfactory. Therefore, the sebum absorbent tapes can be considered to give an acceptable measurement of SERs.

Table II. Amount and Composition of Forehead Skin Surface Lipid (µg/3 h/cm²)

<table>
<thead>
<tr>
<th>Total Lipid</th>
<th>CH</th>
<th>FA</th>
<th>TG</th>
<th>WE + CHE</th>
<th>SQ</th>
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</thead>
<tbody>
<tr>
<td>Absorbent Tapes (left side)</td>
<td>Mean 171.55</td>
<td>2.71</td>
<td>17.87</td>
<td>79.83</td>
<td>47.90</td>
</tr>
<tr>
<td>SD (±)</td>
<td>82.47</td>
<td>1.99</td>
<td>16.89</td>
<td>39.11</td>
<td>28.71</td>
</tr>
<tr>
<td>Hexane Extraction (right side)</td>
<td>Mean 164.00</td>
<td>2.30</td>
<td>18.29</td>
<td>72.23</td>
<td>41.47</td>
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<tr>
<td>SD (±)</td>
<td>78.12</td>
<td>1.48</td>
<td>14.97</td>
<td>39.75</td>
<td>24.68</td>
</tr>
</tbody>
</table>

Key: CH = cholesterol
FA = free fatty acids
TG = triglycerides
WE + CHE = wax and cholesterol esters
SQ = squalene
*µg/cm²/3 h.

Figure 2. Correlation of total lipid collected with sebum absorbent tapes and the hexane extraction technique (r = 0.89).

Figure 3. Separation of cholesterol on TLC with chloroform: ethyl acetate 94:6 (v/v); cholesterol Rf = 0.38. Free fatty acids migrate close to the origin; triglycerides, wax and cholesterol esters, and squalene close to the solvent front.
Results obtained using the sebum absorbent tapes were also in accord with those obtained using the hexane extraction technique of Ruggieri et al [2]. The 2 techniques were in good accord with reference to their efficacy in collection of free fatty acids, triglycerides, wax and cholesterol esters, and squalene from the surface of the skin. The lack of correlation with respect to cholesterol was attributable to interference of the adhesive from the tape with the cholesterol on TLC. In general we have found that this can be avoided by running the last solvent to 15 cm instead of the routine 10 cm distance, giving a good separation of cholesterol from the adhesive. Specific quantitation of the cholesterol could also be achieved by development in chloroform:ethyl acetate, 94:6 (v/v). Alternatively, the adhesive may be completely removed using the sample cleanup procedure described in this communication. In its entirety the cleanup takes only approximately 5 min and is straightforward and easy to perform.

It has recently been demonstrated that a good correlation exists between 1-h measurements of SER [12]. We studied the reproducibility of 3-h measurements of the lipid absorbent tapes for the purpose of comparison with the most widely used methods, which have been based on the standard 3-h SER measurement. However, the reproducibility of the tapes for 1- and 2-h collections of lipid is currently being tested. Another methodologic point concerns the quantitation of lipid; image analysis has been successfully applied to estimation of total lipids on the tapes as an alternative to chromatographic techniques.

We have demonstrated that the sebum absorbent tapes provide a new, reproducible technique for the measurement of SER and quantitation of components of skin surface lipids. Furthermore, the tapes have the advantage over other techniques in that they are convenient to use and do not require the precision that is necessary for many of the existing methods.

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REFERENCES