Stratum Corneum Thickness and Apparent Water Diffusivity: Facile and Noninvasive Quantitation *In Vivo*

Fabrice Pirot,[1,2] Enzo Berardesca,[3] Yogeshvar N. Kalia,[1,4] Malkiat Singh,[5] Howard I. Maibach,[5] and Richard H. Guy[1,4,6]

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INTRODUCTION

As the principal barrier to insensible tissue water loss and to the permeation of topically applied drugs, the stratum corneum (SC) has been the focus of considerable study and research (1). Absolute quantification of permeability parameters across the SC, and their alteration by, for example, dermatological disease or penetration enhancement methodologies, has proved difficult, particularly *in vivo* in man, for many reasons. An obvious problem stems from the fact that the thickness of the SC is not easily determined. Excision and processing for microscopy invariably introduce artifacts, the effects of which on the value of the subsequently deduced thickness remains unknown. In turn, this means that relative changes in the permeability barrier (conveniently measured by alteration in the rate of transepidermal water loss (TEWL)) are also difficult to quantify.

Recently, it was shown that water diffusivity across human SC *in vivo* was independent of position in the membrane, i.e., that transport was homogeneous in this structurally heterogeneous membrane (2). The experimental proof for this deduction rested upon the results of TEWL measurements recorded as a function of repeated adhesive tape-stripping of the SC. Concomitantly, analysis of the data permitted facile determination of SC thickness and the deduction of an apparent average diffusion coefficient for water across the membrane. In this article, the procedure and data analysis developed is applied to a much larger cohort of subjects and a simpler interpretative methodology is then derived to greatly facilitate the experimental demands necessary for quantification of SC thickness and water diffusivity and permeability therein.

[1] Centre Interuniversitaire de Recherche et d'Enseignement, "Pharmapeptides," Parc d'Affaires International, F-74166 Archamps, France.

[2] Laboratoire de Recherche et de Développement de Pharmacie Galénique Industrielle, Université Claude Bernard-Lyon I, 69373 Lyon Cedex 08, France.

[3] Department of Dermatology, University of Parma, IRCCS Policlinico S. Matteo, Pavia, Italy.

[4] Faculté des Sciences—Section de Pharmacie, Laboratoire de Pharmacie Galénique, Université de Genève, CH-1211 Genève 4, Switzerland.

[5] Department of Dermatology, University of California at San Francisco, San Francisco, California 94143.

[6] To whom correspondence should be addressed. (e-mail: rhg@pharmal.cur-archamps.fr)

MATERIALS AND METHODS

Subjects

Thirteen human volunteers (2 male, 11 female), aged from 23 to 60 years (mean 43 years), participated in the study. All were in good general health and had no history of skin disease. The study was approved by the UCSF Committee on Human Research; informed consent was obtained from all subjects.

TEWL and Tape-Stripping

The procedure followed has been fully described in an earlier publication (2). Briefly, after recording an initial TEWL measurement (Servo Med Evaporimeter EP1, Servo Med AB, Kinna, Sweden) on the mid-ventral forearm surface, the SC beneath the observation site was subjected to serial tape-stripping using preweighed 5×4 cm^2 strips of Scotch No. 845 book tape (3M Co., St. Paul, MN); 15 strips in total were removed using a standard application and removal protocol (3–5). TEWL measurements were made after every third tape-strip. The tape-strips were weighed and the average thickness of SC removed was then calculated from the cumulative mass and the SC density of 1 g cm^{-3} (6).

Data Analysis

Fick's 1st Law of Diffusion describes the insensible loss of water ($(TEWL)_o$) across the entire SC (7):

$$(TEWL)_o = \frac{\gamma \cdot \bar{D}}{H} \quad (1)$$

where γ is the SC-viable tissue partition coefficient (K) of water multiplied by the water concentration difference (ΔC) across the membrane, and $\bar{D}$ is the average apparent diffusion coefficient of water in the SC of thickness H. During tape-stripping, the SC is progressively removed (i.e., its thickness is reduced) and TEWL increases, such that Equation (1) must be modified as follows:

$$(TEWL)_X = \frac{\gamma \cdot \bar{D}}{H - x} \quad (2)$$

where $(TEWL)_x$ corresponds to the measured value of TEWL when x μm of SC have been removed. Inversion of Equation (2) gives:

$$(TEWL)_x^{-1} = \frac{H}{\gamma \cdot \bar{D}} - \frac{x}{\gamma \cdot \bar{D}} \quad (3)$$

which predicts that $(TEWL)_x^{-1}$ decreases linearly with increasing x, that the x-intercept of such a plot provides H directly and that, knowing K and ΔC (8), $\bar{D}$ can be determined from either the slope of the line or the y-axis intercept.

Alternatively, given that the linearity of the analysis is established, it is possible to use just the initial TEWL determination together with, for example, that measured after n tape-strips have been removed to obtain H from the solution to

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Table I. Values of SC Thickness (H), and Apparent Water Diffusivity ($\bar{D}$) and Permeability Coefficient K_p Across the SC, for Either (a) a Full Analysis of the Data (left-hand side of the Table), or (b) a Simpler "Two-point" Treatment (right-hand side)

Full analysis (Equation 3)				"Two point" analysis (Equations (1) and (4))		
H (μm)	$10^9\ \bar{D}$ ($cm^2\ s^{-1}$)	$10^7\ K_p$ ($cm\ s^{-1}$)	Subject	H (μm)	$10^9\ \bar{D}$ ($cm^2\ s^{-1}$)	$10^7 K_p$ ($cm\ s^{-1}$)
8.9	3.1	2.1	A	8.1	3.0	2.2
13.9	3.9	1.7	B	12.3	3.6	1.8
14.5	4.2	1.7	C	12.1	3.6	1.8
6.9	2.1	1.8	D	6.6	2.3	2.1
14.6	5.8	2.4	E	8.3	3.1	2.2
22	7.7	2.1	F	21.0	7.9	2.3
18.3	5.8	1.9	G	19.5	7.0	2.2
19.8	7.7	2.3	H	16.4	6.7	2.5
9.8	3.3	2.0	J	8.4	2.9	2.1
13.6	5.8	2.6	K	13.5	5.7	2.5
5.9	2.0	2.0	L	4.7	1.5	1.9
6.4	2.0	1.9	M	8.1	2.9	2.1
9.2	3.6	2.3	N	8.4	3.2	2.3
12.6 ± 5.3	4.4 ± 2.0	2.1 ± 0.3	Mean ± SD	11.3 ± 5.1	4.1 ± 2.0	2.2 ± 0.2

the simultaneous equations represented by Equation (1) and Equation (4):

$$(TEWL)_{x_n} = \frac{\gamma \cdot \bar{D}}{H - x_n} \quad (4)$$

That is,

$$H = \frac{x_n}{1 - R} \quad (5)$$

where x_n is the cumulative SC thickness removed by n tape-strips and $R = (TEWL)_{x_n}/(TEWL)_o$.

RESULTS AND DISCUSSION

Regression analysis of the TEWL measurements as a function of SC thickness removed, in accord with Equation (3), produced highly linear fits (all with $p < 0.05$ and $r > 0.85$). The resulting values of SC thickness (H) obtained from the x-axis intercept and of $\bar{D}$ deduced from the slope of the regression (assuming K = 0.06 (8) and $\Delta C = 1\ g\ cm^{-3}$) are presented in the left-hand part of Table I. The calculated permeability coefficients ($K_p = \bar{D} \cdot K/H$) are also given. The mean values of the three parameters (H = 12.6 (± 5.3) μm, $\bar{D}$ = 4.4 (± 2.0) × 10^{-9} $cm^2\ s^{-1}$ and K_p = 2.1 (± 0.3) × 10^{-7} $cm\ s^{-1}$) compare remarkably well with those determined using only three subjects in the original paper describing this approach (2): 12.7 (± 3.3 μm), 3.83 (± 1.32) × 10^{-9} $cm^2\ s^{-1}$ and 1.84 (± 0.47) × 10^{-7} $cm\ s^{-1}$, respectively.

In the right-hand side of Table I, the values of H, $\bar{D}$ and K_p determined by the simultaneous solution of Equations (1) and (4), using n = 9, are presented. Clearly, despite this somewhat less sophisticated approach, the results are statistically indistinguishable from those produced by the fuller analysis. To emphasize this point, Figure 1 shows the predicted dependencies of TEWL and $(TEWL)^{-1}$ upon SC thickness removed (x) calculated using the corresponding average H and $\bar{D}$ parameters (with K = 0.06 (7) and $\Delta C = 1\ g\ cm^{-3}$) from the two data

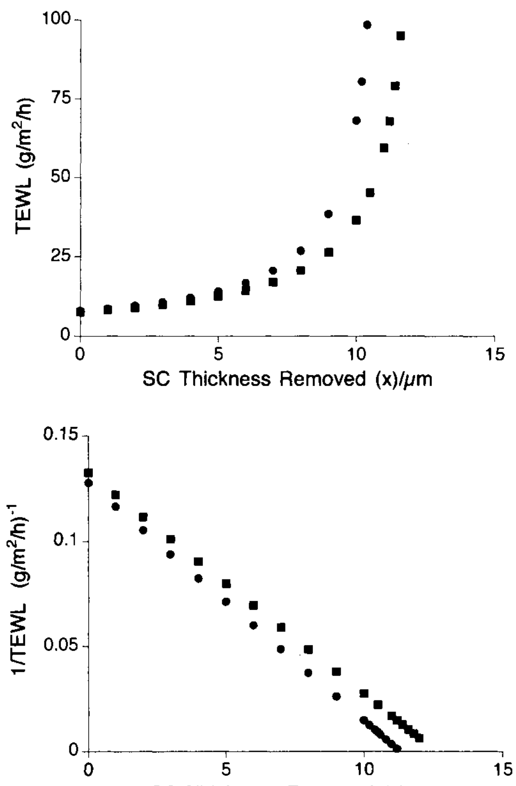

Fig. 1. Prediction of TEWL and $(TEWL)^{-1}$ as a function of SC thickness removed (x) (μm) using the mean values of H and $\bar{D}$ determined by the full data analysis represented by Equation (3) (■) or by the simpler, "two-point" solution of the simultaneous equations, Equations (1) and (4) with n = 9 (●). All data points were calculated assuming K = 0.06 and $\Delta C = 1\ g\ cm^{-3}$.

analysis procedures. Self-evidently, the error introduced by the more facile technique is less than the variability that one can anticipate in a normal cohort of subjects.

To conclude, the results of this study demonstrate that simple, noninvasive two-point measurements of TEWL, and cumulative SC weight removed by tape-stripping, can be used to provide accurate estimates of SC thickness and apparent water diffusivity across the membrane, *in vivo* in man. The results obtained are in good agreement with a more detailed analysis of the data and with previously published values using this and other, less convenient, techniques. While the approach does not permit the molecular mechanism of water diffusion across the SC to be deduced (and hence diffusivity values must be prefaced by the word "apparent"), it does lend itself to facile application at other body sites, and to diseased or (for example) penetration-enhancer perturbed skin.

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