Water Diffusion Characteristics of Human Stratum Corneum at Different Anatomical Sites *In Vivo*

Doris A. Schwindt,[1] Klaus P. Wilhelm,* and Howard I. Maibach

Department of Dermatology, School of Medicine, University of California, San Francisco, California, U.S.A.; *Institute for Applied Dermatological Research, Schenefeld/Hamburg, Germany, and Department of Dermatology, University of Lübeck, Germany

Despite its heterogeneity, stratum corneum (SC) has been described as a homogeneous membrane for water diffusion. We measured water flux across the SC, transepidermal water loss (TEWL), in six women, *in vivo*. At four anatomical sites – back, abdomen, forearm, and thigh – we took measurements during sequential tape stripping. The inverse of TEWL (1/TEWL) and removed SC thickness yielded a highly linear correlation (Pearson's *r* ranging between 0.88 and 0.99). Applying Fick's law of diffusion, we calculated SC thickness (*H*), and SC water diffusion coefficient (*D*). Comparing the results, SC of all women was significantly thicker ($p < 0.05$) at the extremities (12.7 ± 4.2 μm, mean ± SD, $n = 12$) than the abdomen (7.7 ± 1.8 μm, $n = 6$). The calculated diffusion coefficient approximated 2.16 ± 1.14 × 10^{-9} cm^2/s. Compared with the diffusion constant found for SC depleted of lipids, our value was 100-fold lower. In agreement with previous findings that intercellular lipids are a rate determining component of the SC barrier, we suggest that water diffuses mainly through the intercellular space. The calculation of *H* and/or *D*, however, is based on several variables: SC density, the water concentration difference, and the partition coefficient of water between viable epidermis and SC. The literature values vary widely. It is desirable to determine these parameters more precisely, especially if discrete differences, such as between anatomical sites, are to be revealed. ***Key words: stratum corneum thickness/stratum corneum water diffusivity/tape stripping/transepidermal water loss. J Invest Dermatol 111:385–389, 1998***

The stratum corneum (SC), suggested as being approximately 10 μm thick, has been described by the brick and mortar model with the corneocytes being imbedded into lipids (Michaels *et al*, 1975; Elias *et al*, 1983; Elias, 1983). The corneocytes, consisting mainly of keratin, account for most of the SC weight (Eckart, 1989). The lipids are arranged in lamellar sheets, which consist of membrane-like bilayers (Elias *et al*, 1977; Landmann, 1986) of ceramides, cholesterol, and fatty acids (Elias, 1981; Lampe *et al*, 1983). This structure determines SC functionality to act as a barrier: it prevents fluid loss on the one hand, and hinders percutaneous penetration on the other.

This fact would be much less interesting if there were not exceptions to this rule. We know a range of substances that penetrate and/or permeate skin (Bronaugh and Maibach, 1989; Shah and Maibach, 1993). Attempting to solve the question of if, how, and to what extent they penetrate has contributed much to our understanding of SC functionality. One substance that permeates in small amounts is water. The phenomenon that the body constantly loses water independent of the sweat glands is also known as insensible water loss (Benedict and Root, 1926). Water flux across the SC constitutes the largest part of insensible water loss (≈65%; Rothman, 1954; Kuno, 1956) and can be measured as transepidermal water loss (TEWL; Nilsson, 1977). TEWL is one parameter with which to assess the integrity of the skin barrier (Grubauer *et al*, 1989; Elias and Menon, 1991). Corneocytes and intercellular lipids both contribute to prevent water loss, the lipids, however, appear to be the rate determining factor (Potts and Francoeur, 1991). Despite its heterogeneity, Scheuplein (1967a) describes the SC as a membrane for which Fick's laws of passive diffusion are valid (Fickian membrane), thus each layer contributes equally to prevent water loss (Kligman, 1964). This finding was supported by van der Valk and Maibach (1990) and recently confirmed for the forearm by Kalia *et al* (1996).

Regional differences with respect to percutaneous absorption of drugs have been found repeatedly (Feldmann and Maibach, 1967; Wester *et al*, 1984). Variations in lipid composition (Lampe *et al*, 1983) and TEWL measurements have been described (Cua *et al*, 1990). Holbrook and Odland (1974) found the mean thickness and number of cell layers of the SC to vary inter- and intraindividually with anatomic site.

This study measured TEWL *in vivo* during sequential tape stripping at four anatomic sites to calculate the diffusion coefficient of water across the SC and the thickness of the SC. We wanted to confirm the apparent contradiction of a heterogeneous structure behaving like a homogeneous membrane for different anatomic sites.

MATERIALS AND METHODS

Tape stripping procedure Six Caucasian women (age 33.2 ± 3.1 y, mean ± SD) participated after having given informed consent. The study was approved by the University of California Committee on Human Research.

We chose four body areas to evaluate differences in water diffusion and SC thickness: lower back (lumbar region, L2–3 level), abdomen (lower right quadrant), thigh (anterior upper third), and volar forearm (center between cubital fossa and wrist). Each area was stripped consecutively up to 35 times until the stripped area glistened, TEWL exceeded 50 g/(m^2 h), or remained constant.

Manuscript received November 1, 1997; revised May 12, 1998; accepted for publication May 18, 1998.

Reprint requests to: Dr. Howard I. Maibach, Department of Dermatology, School of Medicine, Surge 110, University of California, San Francisco, CA 94143.

Abbreviations: SC, stratum corneum; TEWL, transepidermal water loss.

[1]This study is part of this author's dissertation, University of Lübeck, Germany.

We used Scotch Book Tape (3M, no. 845, adhesive: proprietary synthetic acrylic), cut into pieces of 5.98 ± 0.08 cm^2 (value ± uncertainty). To facilitate handling of the tape strips, we attached a paper punch (0.38 ± 0.03 cm^2) to the sticky surface. Using tweezers, the tape was applied to a site and rubbed lightly to assure adhesion, and after 1 min the strip was removed. Tapes were weighed immediately before application and after removal on a Mettler AT 20 balance (precision 10 μg). TEWL measurements were taken 5 min after every second or third tape strip, depending on the previous change in the corresponding measurements. Room temperature was between 18 and 20°C and relative humidity was between 50% and 55%. TEWL was measured with an evaporation meter (Tewameter TM 210, Courage Khazaka, Cologne, Germany, and Acaderm, Menlo Park, CA) (Distante and Berardesca, 1995). The device evaluates the water vapor pressure gradient above the skin by means of two hygrosensors, located in the open probehead at different heights (Nilsson, 1977). TEWL was recorded continuously and expressed in g/(m^2 h). Mean readings were taken when values had stabilized, after ≈3–4 min.

Data analysis With the experimental procedure described above, the biophysical property TEWL was determined as a function of the cumulative mass of removed SC. Assuming a constant coverage and density of SC on each tape strip, the thickness of the removed SC can be calculated from the cumulative mass:

$$x_i = \frac{m_i}{F\rho} \tag{1}$$

where x_i is the thickness, m_i is the cumulative mass of SC removed by i consecutive tape strips, F is the area of the tape strips (F = 5.6 ± 0.1 cm^2), and ρ is the SC density (set to ρ = 1 g/cm^3; Anderson and Cassidy, 1973).
The first Fickian law describes water diffusion across a homogenous membrane at steady state:

$$\vec{J} = \text{TEWL} = -D\,\frac{\partial c}{\partial x} \tag{2}$$

where $\vec{J}$ is the water flux [given in g/(m^2 h)], D is the diffusion constant or diffusivity (given in cm^2/s), and $\partial c/\partial x$ is the concentration gradient of water. If D is independent of the water concentration, $c(x)$, and if the SC is regarded as a homogeneous membrane with regard to water diffusion, eqn 2 may be written as:

$$\text{TEWL} = -D\,\frac{K\Delta c}{(x-H)} = -D\,\frac{\gamma}{(x-H)} \tag{3}$$

where K is the partition coefficient of water between the viable epidermis and the SC (fixed to K = 0.162; Blank *et al*, 1984), Δc is the finite difference in water concentration between viable epidermis and the surrounding atmosphere (fixed to Δc = 1 g/cm^3; Kalia *et al*, 1996), and H is the overall thickness of the SC. Inversion of eqn 3 leads to:

$$\frac{1}{\text{TEWL}} = \frac{H}{D\gamma} - \frac{x}{D\gamma}$$

As shown previously (Kalia *et al*, 1996), the SC appears to be a homogeneous membrane for water diffusion. In this case a plot of 1/TEWL *versus* x should reveal a linear relationship with an intercept of $H/D\gamma$ and a slope of $1/D\gamma$; therefore D can be calculated from the slope and γ and H from the intercept and slope. Regression analyses were performed with statistical software packages Minitab Ver. 10 (Minitab, State College, PA) and Origin Ver. 4.1 (Microcal Software, Northampton, MA).

RESULTS

For a given skin site and volunteer each tape strip removed approximately the same amount of SC; the first two to three strips, however, tended to remove greater amounts. The number of tape strips strongly correlated with the cumulative mass of removed SC (Pearson's r ranging between 0.88 and 0.99). In the regression analyses (**Fig 1**) the uncertainty of each cumulative mass σ_i was used as its weight factor ($w_i = 1/\sigma_i^2$). The uncertainty increases with the tape strip number i, $\sigma_i = \sqrt{i}\times 10$ μg. Depending on the volunteer and skin site, each strip removes between 120 and 350 μg. This corresponds to a thickness between 0.21 and 0.63 μm (eqn 1).

We used an unweighted regression analysis of 1/TEWL *versus* cumulative mass (proportional to total thickness of stripped SC, x) for each site of every volunteer separately. The advantage of an unweighted regression is that although the first cumulative masses have less error than the total masses derived from later strips, the first TEWL measurements probably have larger errors because steady state might not have been achieved during the time between tape removal and TEWL measurement. A weighted regression would overemphasize the first measurements. Theoretically, about three lag times, $3\tau = (H-x)^2/2D$, should achieve steady state condition (Scheuplein, 1978a), in this case $(H-x) \approx 14$ μm, $D \approx 4\times 10^{-9}$ cm^2/s, $\tau \approx 5$ min, comparable with the time between two TEWL measurements.

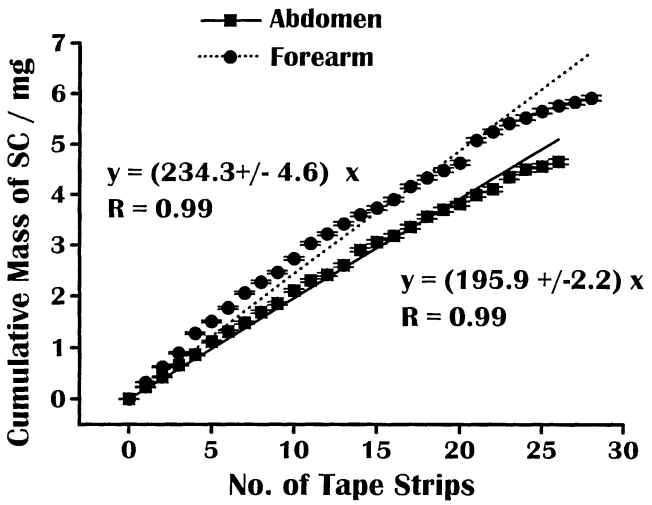

Figure 1. Weighted linear regression analyses (through the origin) for cumulative mass of stripped SC as a function of tape strip number *i* (*error bars*: ± σ). Symbols represent the measurements, and lines represent the regression results. The derived regression functions (slope ± SD) and correlation coefficients for abdomen and forearm of a single volunteer are given. Thus, for a given volunteer and anatomical site each tape strip removed approximately the same mass of SC.

Table I provides the resulting slopes and intercepts of all regression analyses. **Figure 2** depicts the measured data points and the regression lines for volar forearm and abdomen of four volunteers. The intercept of the regression line with the abscissas is the overall SC thickness H. **Table I** summarizes the calculated diffusion coefficients D and overall thicknesses H for all areas of all volunteers. Furthermore, **Table II** gives the results of Friedman's non-parametric analysis of variance by ranks (Portney and Watkins, 1993). The diffusion constant of the SC D was significantly lower for the abdomen compared with the back and forearm [familywise error rate α_{FW} = 0.25, thus with four areas (k = 4) the individual error rate is α = 0.02]. With regard to the thickness H, the SC of the abdomen was significantly thinner than that of the forearm and thigh.

DISCUSSION

Removing SC by sequential stripping with adhesive cellophane tape is a valuable method to determine changes of biophysical skin properties *in vivo*, e.g., TEWL, with decreasing thickness of the SC. The results can provide insight into the structure and function of the SC (Blank, 1953; Tagami *et al*, 1980), and into the penetration of topically applied substances through the SC (Rougier *et al*, 1989). The objective of this study was to determine if there are regional differences in the thickness of and in the water flux across the SC, as the latter is continually removed.

For a quantitative interpretation of tape stripping investigations it is mandatory to know the thickness of removed SC to obtain an accurate profile of the measured property. In the best case the thickness of SC removed with every tape strip is constant, regardless of the amount removed previously. By weighing the tapes before and after stripping the SC *in vivo*, and by performing weighted regression analyses, we showed that the mass of stripped SC was fairly constant for a given anatomical site and volunteer (**Fig 1**) (Bommannan *et al*, 1990). The average mass of stripped SC ranged from 120 to 300 μg. The SD were between 3 and 15 μg, approximately equal to the experimental

Table I. Water diffusion coefficients *D* and total stratum corneum thicknesses *H*[a]

Volunteer	Site	Pearson's *r*	10^3 Slope (10^3 m^2 h/g^2)	Intercept (m^2 h/1000g)	10^9 *D* (cm^2/s)	*H* (μm)
A	back	–0.943	–12.0 ± 1.2	97.8 ± 4.4	2.6 ± 1.8	14.6 ± 4.3
B	back	–0.940	–7.97 ± 0.75	63.5 ± 1.8	3.8 ± 2.6	14.2 ± 4.2
C	back	–0.958	–16.4 ± 1.6	80.6 ± 3.4	1.8 ± 1.3	8.8 ± 2.6
D	back	–0.978	–15.9 ± 1.1	84.1 ± 2.5	2.0 ± 1.3	9.4 ± 2.7
E	back	–0.984	–13.41 ± 0.65	69.7 ± 1.1	2.3 ± 1.6	9.3 ± 2.6
F	back	–0.881	–20.4 ± 3.2	122.2 ± 7.7	1.5 ± 1.0	10.7 ± 3.5
A	forearm	–0.982	–12.77 ± 0.68	106.5 ± 2.6	2.4 ± 1.6	14.9 ± 4.2
B	forearm	–0.983	–21.4 ± 1.2	81.5 ± 2.1	1.4 ± 1.0	6.8 ± 1.9
C	forearm	–0.975	–6.65 ± 0.38	61.4 ± 1.1	4.6 ± 3.1	16.5 ± 4.7
D	forearm	–0.981	–9.58 ± 0.54	76.5 ± 1.8	3.2 ± 2.2	14.3 ± 4.0
E	forearm	–0.924	–16.3 ± 1.9	90.2 ± 3.7	1.9 ± 1.3	9.9 ± 3.0
F	forearm	–0.957	–18.0 ± 1.5	116.2 ± 4.5	1.7 ± 1.2	11.6 ± 3.4
A	abdomen	–0.978	–19.9 ± 1.2	109.4 ± 3.6	1.5 ± 1.0	9.8 ± 2.8
B	abdomen	–0.985	–26.8 ± 1.4	98.1 ± 2.6	1.14 ± 0.8	6.5 ± 1.8
C	abdomen	–0.908	–15.5 ± 2.0	76.6 ± 3.0	2.0 ± 1.4	8.8 ± 2.7
D	abdomen	–0.991	–18.69 ± 0.81	95.9 ± 1.9	1.7 ± 1.1	9.2 ± 2.6
E	abdomen	–0.941	–27.1 ± 2.8	98.7 ± 4.2	1.13 ± 0.8	6.5 ± 1.9
F	abdomen	–0.996	–36.08 ± 0.97	110.9 ± 1.6	0.85 ± 0.6	5.5 ± 1.5
A	thigh	–0.992	–21.18 ± 0.82	108.0 ± 2.3	1.45 ± 1.0	9.1 ± 2.6
B	thigh	–0.906	–8.5 ± 1.0	82.0 ± 3.3	3.6 ± 2.5	17.2 ± 5.2
C	thigh	–0.947	–5.97 ± 0.54	67.9 ± 2.2	5.1 ± 3.5	20.3 ± 5.9
D	thigh	–0.989	–27.7 ± 1.1	198.6 ± 4.6	1.12 ± 0.8	12.8 ± 3.6
E	thigh	–0.979	–18.0 ± 1.0	100.9 ± 2.6	1.7 ± 1.2	10.0 ± 2.8
F	thigh	–0.995	–25.49 ± 0.71	129.6 ± 1.7	1.2 ± 0.8	9.1 ± 2.5

[a]As calculated from linear regression of 1000/TEWL versus mass stripped stratum corneum *m*. Slopes, intercepts, and Pearson's correlation coefficients of the regression analyses for four anatomical regions are given. The errors given behind the slopes and intercepts are one SD. The errors behind *D* and *H* are their uncertainties as calculated from error propagation of eqn 4. The following values and their corresponding uncertainties have been used: $\Delta c = (1.0 \pm 0.05)$ g/cm^3, $K = 0.16 \pm 0.1$, $\rho = (1.0 \pm 0.3)$ g/cm^3. One microgram of stripped stratum corneum on a tape area of 5.6 ± 0.1 cm^2 corresponds to a thickness of $1.79 \pm 0.5 \times 10^{-3}$ μm.

Table II. Friedman's two-way analysis of variances by ranks[a]

	10^9 *D* (cm^2/s)			*H* (μm)			
Area	Estimated median	Sum of ranks	Observed mean ± SD	Estimated median	Sum of ranks	Observed mean ± SD	Reference[b]
Back	2.32	19.0	2.34 ± 0.82	12.17	14.5	11.2 ± 2.6	9.4 (8.2–11.3)
Forearm	2.30	19.0	2.54 ± 1.2	13.5	20.0	12.3 ± 3.6	12.9 (8.1–16.2)
Thigh	1.92	13.0	2.37 ± 1.6	13.05	18.0	13.1 ± 4.7	10.9 (7.7–15.3)
Abdomen	1.46	9.0	1.38 ± 0.42	8.74	7.5	7.7 ± 1.7	8.2 (6.9–9.8)
	$\chi^2 = 7.2$, p = 0.066 Grand median = 2.0				$\chi^2 = 9.2$, p = 0.027 Grand median = 11.87		

[a]The *D* and *H* values are given in Table I. The critical rank difference for a post hoc comparison between any two areas is 9.12.
[b]Holbrook and Odland (1974): given are the mean stratum corneum thicknesses ($n = 6$) and in parentheses the range of thicknesses.

precision of the balance (10 μg), which supports the validity of the regression analyses.

The relationship between 1/TEWL and the total mass of removed SC, *m*, was linear for all anatomical sites (**Fig 2, Table I**). Therefore, it appears that the SC acts *in vivo* as a Fickian membrane for water diffusion at steady state, confirming the results of Kalia *et al* (1996) from the volar forearm.

To calculate the diffusion constant *D* from the slope ($= 1/K\Delta cD$), and the overall SC thickness *H* from the ratio between intercept ($= H$ slope) and the slope, requires several variables: the water partition coefficient, *K*, between viable epidermis and the SC, as well as the difference in water concentration between viable epidermis and the surrounding atmosphere, Δc, and/or the density of the SC, ρ. These parameters have to be assumed to be constant within one and between sites. The values for ρ in the literature range from 0.8 to 1.4 g/cm^3 (Scheuplein, 1967b; Anderson and Cassidy, 1973). More controversial are the partition coefficients *K*. Early investigations determine *K in vitro* as a bulk property between a water phase and SC samples, with *K* being a strong function of the ambient water content ($K = 0.162$ for 60% relative humidity; Blank *et al*, 1984). Potts and Francoeur (1991) give $K = 0.06$ for water partition into the intercellular lipids only. Kalia *et al* (1996) used this value in their calculations. The result of Potts and Francoeur (1991) is based on two assumptions about intercellular lipids: firstly that their on average mass is 15% of dried SC, and secondly that their average relative molar weight is 500 u. Moreover, they assume that *K* is independent of the ambient water content. Water uptake (or loss) of SC increases linearly with increasing ambient water content, and above 80% relative humidity it increases strongly non-linearly (Blank *et al*, 1984; Takenouchi *et al*, 1986). We believe that water partitioning *in vivo* is a bulk property of the entire SC, although it is likely that the predominant pathway of water diffusion is through the intercellular lipids. Therefore, we used $K = 0.162$ for a reasonable ambient relative humidity of 60% to calculate the diffusion coefficient given in **Table I**. Error analyses indicate that the uncertainty in the partition coefficient *K* of $\Delta K = \pm 1$ contributes $\Delta D/D = 62\%$. The uncertainty in SC density $\Delta\rho = \pm 0.3$ g/cm^3 contributes $\Delta D/D = 28\%$. Thus, without taking into account any error due to Δc, ΔD is about as large as *D* itself, degrading the determination of *D* to an order of magnitude estimation. The situation is different for the calculation of SC thickness. The error ΔH is

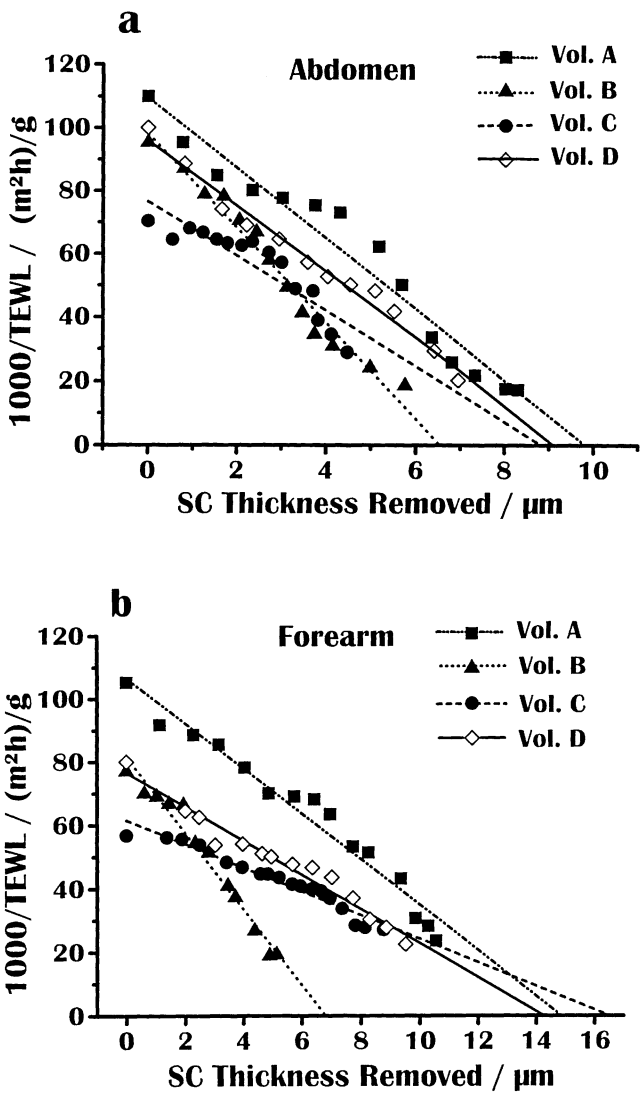

Figure 2. Linear regression analyses of 1000/TEWL *versus* thickness of stripped SC x for the abdomen (*a*) and volar forearm (*b*). Symbols represent the measurements, and lines represent the regression results. The linearity confirms that human SC may be regarded as a Fickian membrane for water diffusion.

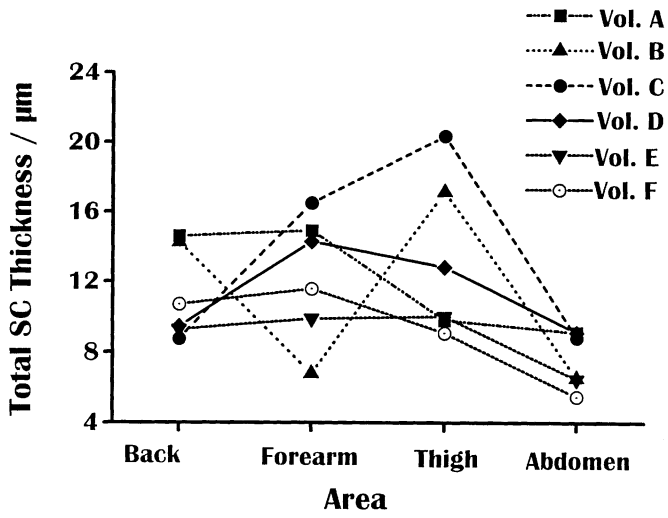

Figure 3. Regional variations of SC thickness H for six volunteers.

predominated by the uncertainty of SC density $\Delta\rho$ only, resulting in $\Delta H/H = 28\%$.

The calculated SC thicknesses (**Table I**) range between 5 and 20 µm. These results agree reasonably well with values derived from ultrastructural investigations of Holbrook and Odland (1974) (**Table II**), thus the diffusion pathlength is approximately equal to the physical thickness of the SC. In contrast to Potts and Francoeur (1991) (H = 880 µm), we found no indication of an effectively tortuous pathway for water, diffusing across the SC.

Comparing different anatomical sites we found that SC of the extremities was significantly thicker than that of the abdomen, which is in accord with data of Holbrook and Odland (1974) (**Table II**).

The results derived from the data of all women reveal interindividual variation. As discussed above, one major uncertainty in the calculation of H and D is due to the uncertainty in SC density ρ. If the density varies between subjects, this will not enhance the differences between anatomical sites. On the other hand, the density might vary regionally within subjects. In this case, introducing density values that account for this regional variation might result in clearer differences in SC thickness.

To account for regional variations in percutaneous absorption, Guy and Maibach (1985) constructed penetration indices. With the forearm set to 1.0, the trunk is indexed between 2.5 and 3.0. These penetration indices correlate negatively with the SC thicknesses (**Table II**). Rougier *et al* (1986) found the total penetration of benzoic acid through abdominal skin to be higher than that through the skin of the back, arm, and thigh. Bronaugh (1985) reported the permeability of male rat skin to be higher at the abdomen compared with the back. The skin thickness of these animals is less for the abdomen than for the back. Thus, SC thickness may be regarded as one factor contributing to the regional variation in percutaneous absorption. The permeability of palmar and plantar skin, however, seems to differ from the sites discussed above. Despite a SC that is 20–50 times thicker, its permeability is similar to that of the forearm (Feldmann and Maibach, 1967; Scheuplein, 1978b). Therefore these sites are special cases regarding the relation between permeability and SC thickness.

The question why the SC appears to act as a homogenous membrane for water diffusion, despite its heterogeneous structure, remains puzzling. Thinking of the SC in the brick and mortar model with the corneocytes as the bricks and the intercellular lipids as the mortar, it is tempting to attribute the observed homogeneity to the intercellular lipids as the more continuous domain. Experimental evidence from model lipids of the SC shows a 100-fold increased water diffusion coefficient D_{lip} compared with SC *in vitro* D_{SC} (Friberg and Kayali, 1989). In relation to water self diffusion $D_{self} \approx 3 \times 10^{-5}$ cm^2/s (Andrussow and Schramm, 1969), the following relationship seems to hold:

$$D_{self} = 10^2\, D_{lip} = 10^4\, D_{SC}$$

This supports the idea that intercellular lipids determine water diffusion through the SC; the corneocytes appear to be minimally permeable. Further experimental data are required to support this mechanism. One approach could be to extract the intercellular lipids *in vitro*. As described by Onken and Moyer (1963) this will lead to an increase of TEWL. Analogously to the experiment by Fridberg *et al* (1990), who added model lipids to the previously depleted SC, one could refill the volume with substances of known water diffusivity. Thereafter the water diffusion constant for this refilled SC would be measured. If the ratios between the diffusion coefficients of pure substance and the refilled SC were constant for several substances, this would be a strong indication that water diffusion through the corneocytes is negligible.

Although the route of water diffusing across the intercellular lipids is twisted, the calculated diffusion pathlength H was equal to the physical thickness of the SC. The reason is that the measured water flux is limited by vertical water diffusion through the intercellular lipids and not by lateral diffusion. In other words, only the vertical component of the diffusion path contributes to the calculated overall diffusion pathlength H (Pallett *et al*, 1997).

REFERENCES

Anderson RL, Cassidy JM: Variations in physical dimensions and chemical composition of human stratum corneum. *J Invest Dermatol* 61:30–32, 1973

Andrussow L, Schramm B: Eigenschaften der Materie in ihren Aggregatszustanden. In: Schäfer K (ed.). *Transportphdnomene I (Viskositdt und Diffusion)*. Berlin: Springer, 1969, p. 5

Benedict FG, Root HF: Insensible perspiration: its relation to human physiology and pathology. *Arch Intern Med* 38:1–35, 1926

Blank IH: Further observations on factors which influence the water content of the stratum corneum. *J Invest Dermatol* 21:259–269, 1953

Blank IH, Moloney III J, Emslie AG, Simon I, Apt C: The diffusion of water across the stratum corneum as a function of its water content. *J Invest Dermatol* 82:188–194, 1984

Bommannan D, Potts RO, Guy RH: Examination of stratum corneum barrier function in vivo by infrared spectroscopy. *J Invest Dermatol* 95:403–408, 1990

Bronaugh RL: Determination of percutaneous absorption by in vitro techniques. In: Bronaugh RL, Maibach HI (eds). *Percutaneous Absorption*. New York: Dekker, 1985, pp. 267–279

Bronaugh RL, Maibach HI (eds): *Percutaneous Absorption: Mechanisms-Methodology-Drug Delivery*, 2nd edn. New York: Dekker, 1989

Cua A, Wilhelm K-P, Maibach HI: Cutaneous sodium lauryl sulphate irritation potential: age and regional variability. *Br J Dermatol* 123:607–613, 1990

Distante F, Berardesca E: Transepidermal water loss. In: Berardesca E, Elsner P, Wilhelm K-P, Maibach HI (eds). *Bioengineering of the Skin: Methods and Instrumentation*. Boca Raton: CRC Press, 1995, pp. 1–4

Eckart RL: Structure, function and differentiation of the keratinocyte. *Physiol Rev* 69:1316–1345, 1989

Elias PM: Lipids and the epidermal permeability barrier. *Arch Dermatol Res* 270:95–117, 1981

Elias PM: Epidermal lipids, barrier function, and desquarnation. *J Invest Dermatol* 80:44s–49s, 1983

Elias PM, Menon GK: Structural and lipid biochemical correlates of the epidermal permeability barrier. *Adv Lipid Res* 24:1–26, 1991

Elias PM, Goerke J, Friend DS: Mammalian epidermal barrier layer lipids: composition and influence on structure. *J Invest Dermatol* 69:535–546, 1977

Elias PM, Grayson S, Lampe MA, Williams ML, Brown BE: The intercomeocyte space. In: Marks R, Plewig G (eds). *Stratum Corneum*. Berlin: Springer-Verlag, 1983, pp. 53–67

Feldmann RJ, Maibach HI: Regional variation in percutancous penetration of ^{14}C Cortisol in man. *J Invest Dermatol* 48:181–183, 1967

Friberg SE, Kayali I: Water evaporation rates from a model of stratum corneum lipids. *J Pharm Sci* 78:639–643, 1989

Friberg SE, Kayali I, Beckerman W, Rhein LD, Simion A: Water permeation of reaggregated stratum comeurn with model lipids. *J Invest Dermatol* 94:377–380, 1990

Grubauer G, Feingold KR, Harris RM, Elias PM: Lipid content and lipid type as determinants of the epidermal permeability barrier. *J Lipid Res* 30:89–96, 1989

Guy RH, Maibach HI: Calculations of body exposure from percutancous absorption data. In: Bronaugh RL, Maibach HI (eds). *Percutaneous Absorption*. New York: Dekker, 1985, pp. 461–466

Holbrook KA, Odland GF: Regional differences in the thickness (cell layers) of the human stratum comeum: an ultrastructural analysis. *J Invest Dermatol* 62:415–422, 1974

Kalia YN, Pirot F, Guy RH: Homogeneous transport in a heterogeneous membrane: water diffusion across human stratum corneum in vivo. *Biophys J* 71:2692–2700, 1996

Kligman AN: The biology of the stratum corneum. In: Montagna W, Lobitz WC (eds). *The Epidermis*. New York: Academic Press, 1964, pp. 387–433

Kuno Y: *Human Perspiration*. Springfield, IL: CC Thomas, 1956

Lampe MA, Burlingname AL, Whitney JA, Williams ML, Brown BE, Roitman E, Elias PM: Human stratum corneurn lipids: characterization and regional variations. *J Lipid Res* 24:120–130, 1983

Landmann L: Epidermal permeability barrier: transformation of lamellar-granule disks into intercellular sheets by a membrane-fusion process: a freeze-fracture study. *J Invest Dermatol* 87:202–209, 1986

Michaels AS, Chandrasakaran SK, Shaw X: Drug permeation through human skin: theory and in vitro experimental measurement. *Aiche J* 21:985–996, 1975

Nilsson GE: Measurement of water exchange through skin. *Med Biol Eng Comput* 15:209–218, 1977

Onken HD, Moyer CA: The water barrier in human epidermis. *Arch Dermatol* 87:584–590, 1963

Pallett MA, Watkinson AC, Hadgraft J, Brain KR: Comparison of permeability data from traditional diffusion cells and ATR-FTIR spectroscopy. Part H. Determination of diffusional pathlengths in synthetic membranes and human stratum corneum. *Int J Pharm* 154:217–227, 1997

Portney Gross L, Watkins MP: *Foundations of Clinical Research, Applications to Practice*. Norwalk, CT: Appleton & Lange, 1993

Potts RO, Francoeur ML: The influence of stratum corneurn morphology on water permeability. *J Invest Dermatol* 96:495–499, 1991

Rothman S: Insensible water loss. In: *Physiology and Biochemistry of the Skin*. Chicago: University of Chicago Press, 1954, pp. 233–243

Rougier A, Dupuis D, Lotte C, Roquet R, Wester RC, Maibach HI: Regional variation in percutancous absorption in man: measurement by the stripping method. *Arch Dermatol Res* 278:465–469, 1986

Rougier A, Lotte C, Maibach HI: In vivo relationship between percutaneous absorption and transepidermal water loss. In: Bronaugh RL, Maibach HI (eds). *Percutaneous Absorption: Mechanisms-Methodology-Drug Delivery.* New York: Dekker, 1989, pp. 175–190

Scheuplein RJ: Mechanism of percutaneous absorption, II. Transient diffusion and the relative importance of various routes of skin penetration. *J Invest Dermatol* 48:79–88, 1967a

Scheuplein RJ: Molecular structure and diffusional processes across intact epidermis. Contract DA 18–108-AMC 148 (A), Edgewood Arsenal, MD: Medical Research Laboratory, 1967b

Scheuplein RJ: Skin permeation. In: Jarret A (ed.). *The Physiology and Pathophysiology of the Skin*. New York: Academic Press, 1978a, pp. 1693–1730

Scheuplein RJ: Site variations in diffusion and permeability. In: Jarret A (ed.). *The Physiology and Pathophysiology of the Skin*. New York: Academic Press, 1978b, pp. 1731–1752

Shah VP, Maibach HI (eds): *Topical Drug Bioavailability, Bioequivalence and Penetration*. New York: Plenum Press, 1993

Tagami FI, Ohi M, Iwatsuki K, Kanamaru Y, Yamada M, Ichijo B: Evaluation of the skin surface hydration in vivo by electrical measurement. *J Invest Dermatol* 75:500–507, 1980

Takenouchi M, Suzuki H, Tagami H: Hydration characteristics of pathologic stratum corneum – evaluation of bound water. *J Invest Dermatol* 87:574–576, 1986

van der Valk PGM, Maibach HI: A functional study of the skin barrier to evaporative water loss by means of repeated cellophane-tape stripping. *Clin Exp Dermatol* 15:180–182, 1990

Wester RC, Maibach HI, Bucks DAW, Aufrere MB: In vivo percutaneous absorption of paraquat from hand, leg and foreann of humans. *J Toxicol Environ Health* 14:759–762, 1984