Biophysical Journal Volume 71 November 1996 2692–2700

Homogeneous Transport in a Heterogeneous Membrane: Water Diffusion Across Human Stratum Corneum In Vivo

Yogeshvar N. Kalia, Fabrice Pirot, and Richard H. Guy
Departments of Biopharmaceutical Sciences and Pharmaceutical Chemistry, University of California–San Francisco, San Francisco, California 94143-0446 USA

ABSTRACT The objective of this study was to determine whether a structurally heterogeneous biomembrane, human stratum corneum (SC), behaved as a homogeneous barrier to water transport. The question is relevant because the principal function of the SC in vivo is to provide a barrier to the insensible loss of tissue water across the skin. Impedance spectra (IS) of the skin and measurements of the rate of transepidermal water loss (TEWL) were recorded sequentially in vivo in human subjects as layers of the SC were progressively removed by the serial application of adhesive tape strips. The low-frequency ($\leq$100 rad s^{-1}) impedance of skin was much more significantly affected by tape stripping than the higher frequency values; removal of the outermost SC layer had the largest effect. In contrast, TEWL changed little as the outer SC layers were stripped off, but increased dramatically when 6–8 μm of the tissue had been removed. It follows that the two noninvasive techniques probe SC barrier integrity in somewhat different ways. After SC removal, recovery of barrier function, as assessed by increasing values of the low-frequency impedance, apparently proceeded faster than TEWL decreased to the prestripping control. The variation of TEWL as a function of SC removal behaved in a manner entirely consistent with a homogeneous barrier, thereby permitting the apparent SC diffusivity of water to be found. Skin impedance (low frequency) was correlated with the relative concentration of water within the SC, thus providing an in vivo probe for skin hydration. Finally, the SC permeability coefficient to water, as a function of SC thickness, was calculated and correlated with the corresponding values of skin admittance derived from IS.

INTRODUCTION

Transport across the stratum corneum (SC), the outermost 15–20 cell layers of the epidermis, is believed to be the rate-determining step for the passage of most molecules across skin. The stratum corneum contains flattened keratinocytes embedded in a multilamellar lipid matrix. The SC lipids are thought to be the primary barrier against water loss (Williams and Elias, 1987; Wertz et al., 1987; Golden et al., 1987; Potts and Francoeur, 1990). The keratinocyte aspect ratio increases the tortuosity of the predominantly intercellular route of transport and explains, at least in part, the highly efficient barrier function properties (Potts and Francoeur, 1991). In addition to being responsible for controlling water loss, removal of the stratum corneum drastically reduces skin impedance, leading to the deduction that the intrinsic opposition to current flow across the skin also resides in this tissue layer (Tregear, 1966). Equivalent circuits have modeled the SC as a parallel arrangement of a resistor, representing essentially aqueous transport pathways, and either a capacitor or a more sophisticated circuit element, representing the lipid milieu, which is suggested as being the source of the reactive (predominantly capacitative) contribution (Tregear, 1966; Yamamoto and Yamamoto, 1976; McAdams and Jossinet, 1991; Kontturi and Murtomäki, 1994; Kalia and Guy, 1995).

The initial aim of our investigation was to use impedance spectroscopy (IS) and transepidermal water loss (TEWL) to establish whether the SC, a heterogeneous membrane, behaved as a homogeneous barrier to water transport and, if so, to determine the value of the diffusivity of water across the SC in vivo. Another objective was to deduce an impedance profile of the SC as a function of depth and to correlate this to the water concentration profile across the SC; in essence, we posed the question, "Do all stratum corneum layers contribute equally to the total membrane impedance?" Furthermore, is the impedance-depth relationship correlated with the corresponding dependence of TEWL, the classic measure of barrier integrity, as successive layers of the stratum corneum are removed? It has been shown that, during destruction of the stratum corneum barrier by sequential tape stripping (Wolf, 1939; Pinkus, 1951; van der Valk and Maibach, 1990), removal of the deeper layers of the stratum corneum is necessary to cause a significant increase in TEWL (van der Valk and Maibach, 1990). The in vivo tape stripping data (van der Valk and Maibach, 1990) were analyzed successfully by assuming Fickian diffusion, but the diffusivity of water was not determined. Blank and co-workers measured tritiated water flux in vitro and calculated the permeability and diffusion coefficients by using a Fickian model (Blank et al., 1984) and projected their data to the in vivo situation. Liron and co-workers studied the kinetics of water vapor absorption-desorption from porcine SC and estimated diffusion coefficients from the non-steady-state solution of Fick's Second Law (Liron

---

Received for publication 5 June 1996 and in final form 12 August 1996.

Address reprint requests to Drs. Yogeshvar N. Kalia and Richard H. Guy, Centre Interuniversitaire de Recherche et d'Enseignement, "Pharmapeptides," Campus Universitaire, Parc d'Affaires International, F-74166 Archamps, France. Tel.: (33)-04-50-31-55-30; Fax: (33)-04-50-95-28-32; E-mail: guy@sc2a.unige.ch.

0006-3495/96/11/2692/09 $2.00

et al., 1994). Previous attempts have been made to correlate skin impedance with skin hydration; however, once again, inferences about the in vivo situation had to be extrapolated from in vitro data (Obata and Tagami, 1989).

The recovery of skin barrier function was monitored over a period of several days, again using TEWL (Frodin and Skogh, 1984) and IS to identify any differences between the two probe techniques. The rates at which both properties returned to their basal values were compared and interpreted mechanistically.

MATERIALS AND METHODS

Chemicals

HEPES buffer and NaCl were obtained from Sigma Chemical Company (St. Louis, MO). Deionized water (resistivity $\geq$ 18 MΩ cm^{-1}) that had been purified by a Millipore System (Milli-Q UFplus; Millipore, Bedford, MA) was used to prepare all solutions.

Electrodes

The alternating current, required for the impedance measurements, was applied using Tendertrace gel adhesive Ag/AgCl electrodes (NDM, Dayton, OH). The area of the gel was 6 cm^2, of which the electrode surface occupied 0.79 cm^2.

Experimental apparatus

A Macintosh Quadra 800 (Apple Computers, Cupertino, CA) running LabVIEW 3.0.1 (National Instruments, Austin, TX) was used to control a signal generator (HP8116A Pulse/Function Generator; Hewlett-Packard Co., North Hollywood, CA). At an applied voltage of 1.0 V (peak to peak), this produced a sinusoidal alternating current, the frequency of which was raised from 1 Hz to 1 kHz incrementally, with 10 frequency points sampled per decade. The electrical circuit used for the impedance measurements included a 2-MΩ resistor in series with the skin. Thus at the applied voltage of 1.0 V (peak to peak), the sinusoidal current remained approximately constant (~0.2 μA). The potential difference across the skin was measured using a lock-in amplifier (model SR850 DSP; Stanford Research Instruments, Sunnyvale, CA). An isolation transformer (Professional Design and Development Services, Berkeley, CA) protected the human subject by ensuring complete electrical separation of the subject from the main power supply.

TEWL measurements were made in the usual way using a Servo Med Evaporimeter EP1 (Servomed AB, Stockholm, Sweden) (Frodin and Skogh, 1984; Pirot et al., 1995).

Subjects

Three human volunteers (two male, one female), aged from 27 to 29 years, participated in the study. All subjects were in good general health and had no history of dermatological disease. Informed consent was obtained from all participants. The study was approved by the UCSF Committee on Human Research.

Experimental procedure

Monitoring TEWL and impedance as a function of SC depth

After recording an initial TEWL measurement, two gel adhesive electrode pads were placed on the subject's forearm, approximately 2–3 mm apart (corresponding to a separation between the active electrodes of 1.5 cm), and an initial impedance spectrum was recorded. The skin surface that had been covered by the electrode pads and the intervening space between were then subjected to serial tape stripping with preweighed 5 × 4 cm strips of Scotch no. 845 book tape (3M, St. Paul, MN); between 20 and 30 tape strips were removed. The tape strips were placed on the forearm surface and pressed down to produce a uniform adhesion to the skin. Subsequently, the strip was removed in a single continuous motion, thereby removing a relatively uniform layer of stratum corneum. TEWL and IS measurements were made after every second tape strip. Complete removal of the stratum corneum was deemed to have occurred when the TEWL measurement reached 80–100 g m^{-2} h^{-1}, or when a constant reading was obtained. The tape strips were weighed and the cumulative amount and thickness of SC removed were calculated, assuming a density of 1 g cm^{-3} and uniform coverage of SC on the tape strip. The density of skin has previously been determined (Anderson and Cassidy, 1973) and reported to be in the range of 0.8–1.3 g cm^{-3}. The skin was not prepared in any way before the measurements; each subject refrained from using any skin preparations (moisturizers, etc.) on the day of the experiment.

Monitoring recovery of skin barrier function

IS and TEWL measurements were recorded at 24-h intervals at the tape-stripped site for a period of 3–8 days. The gradual increase in skin impedance and the return to basal TEWL values enabled the recovery of a functional stratum corneum to be determined.

RESULTS

The impedance (*Z*) of the stratum corneum as a function of angular frequency and increasing skin depth is shown in Fig. 1. The initial tape strips were found to remove thicker layers of the stratum corneum, each strip removing approximately 0.5–0.6 μm. Subsequently, however, this value

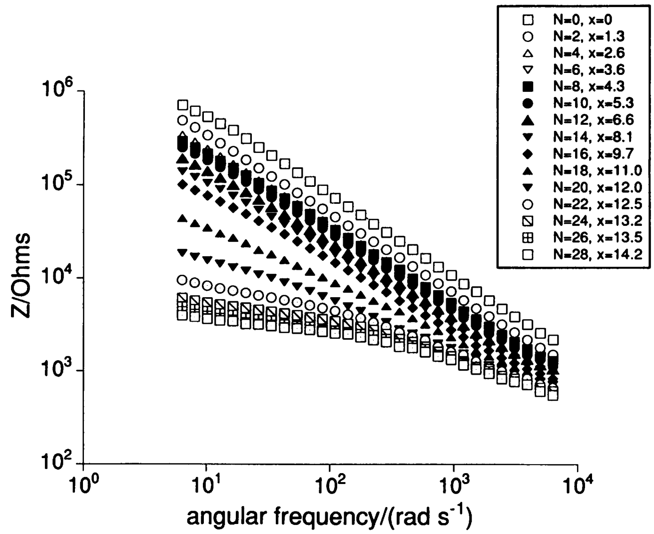

FIGURE 1 Skin impedance measured as a function of angular frequency during stratum corneum removal by serial tape-stripping. The large decrease in the magnitude of the impedance, as successive skin layers are removed, requires the use of a logarithmic scale. *N* = tape-strip number, and *x* = SC thickness removed (μm) as determined from the cumulative weight of SC removed by the tape strips. Impedances shown in this and subsequent figures are given per unit area (cm^2). Angular frequency (ω) = 2π × frequency (Hz). Data from Subject B.

decreased to 0.2–0.3 μm per tape strip. The tape strip number at which this transition occurred varied between subjects, although the trend was consistently observed. The looser packing and concomitant ease of removal of the stratum corneum at the skin surface (King et al., 1979) have been attributed to the decrease in the number of desmosomes (Chapman and Walsh, 1990; Chapman et al., 1991; Downing, 1992) and presumably explain the presence of thicker tissue layers on the initial tape strips. At any given skin depth, the impedance decreased with increasing frequency. Although removal of each successive layer caused skin impedance to decrease across the entire frequency range, the effect was most pronounced at lower frequencies. This can be rationalized as follows: our previous work showed that impedance changes induced by iontophoresis manifested themselves at lower frequencies (Kalia and Guy, 1995); impedance decreases as ions enter resistive pathways in the stratum corneum under the influence of the applied potential; tape stripping facilitates easier access to these transport routes by physically removing the constraint imposed on ion flow by the outer SC layers. Once the innermost (more aqueous) layers had been reached, the impedance response became flatter, essentially resistive, with much less variation across the frequency spectrum. The tape stripping process removed the SC lipid matrix, which is the putative source of the reactive (essentially capacitative) impedance for SC (Kalia and Guy, 1995).

Fig. 2 shows the effect of stratum corneum removal on the phase angle associated with the impedance. In general, the phase angle at a given frequency decreases with progressive tape stripping. This suggests that removal of SC layers decreases capacitative current flow, which is the dominant route at higher frequencies in unperturbed stratum corneum. Again, this is consistent with the hypothesis that the SC lipid-protein domains are responsible for the membrane's capacitance.

Fig. 3 shows the relative impedance (i.e., impedance at frequency ω at depth d in the SC divided by impedance at frequency ω at $d = 0$, $Z_d(\omega_i)/Z_{d=0}(\omega_i)$) as a function of tape strip number. The data emphasize that SC impedance decreased dramatically when the outer layers were removed. Subsequent layers formed apparent clusters of four to eight layers with similar impedance values.

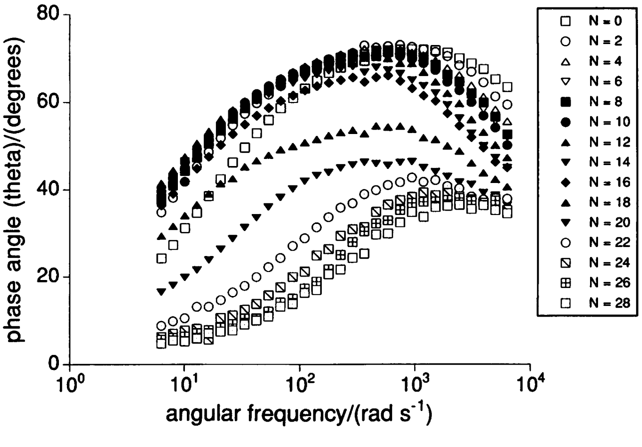

FIGURE 2 Phase angle, θ, at a given frequency decreased with progressive removal of SC layers (N = tape-strip number). Data from Subject B.

The impedance locus changed as measurements were taken at progressively greater depths within the SC (Fig. 4) (Yamamoto and Yamamoto, 1976). The loci were depressed. We note further that, because there was no equilibration period after electrode application on the skin before commencing measurements, the initial loci displayed no characteristic curvature at the low frequency limit (Kalia and Guy, 1995). As the lower layers were reached, more complex loci were observed and the impedance at low frequencies attained an almost constant value of Z_{Imag}.

Baseline TEWL rates were less than 10 g m^{-2} h^{-1}. Progressive tape stripping caused TEWL to increase 18-fold, 7.4-fold, and 6.8-fold, respectively, in the three subjects studied (Table 1). A fixed number of tape strips resulted in neither the same net change in TEWL nor an equivalent amount of SC being removed. For example, after 20 tape strips, SC to a depth of 9, 12.0, and 9.8 μm had been removed from subjects A, B, and C, respectively, whereas their corresponding TEWL values had risen to 89.5 g m^{-2} h^{-1} (from the control of 5.6 g m^{-2} h^{-1}), 29.2 g m^{-2} h^{-1} (from 5.7 g m^{-2} h^{-1}), and 44.6 g m^{-2} h^{-1} (from 8.6 g m^{-2} h^{-1}). Removal of the initial tape strips had a smaller proportionate effect on TEWL than removal of the underlying layers. It was only after the removal of approximately 6–8 μm of SC tissue that steeper increases in TEWL were seen.

Fig. 5 illustrates the inverse correlation between TEWL and impedance (at an angular frequency of 10.12 rad s^{-1}) as a function of thickness of SC removed. Both quantities are normalized by dividing the value at a given depth by the basal value. The data clearly display that removal of the outermost SC layers affected impedance more than TEWL (vide infra). After removal of approximately 4 μm of SC, the impedance had decreased to approximately 40% of the basal value, whereas no appreciable differences in TEWL were observed until removal of deeper SC layers. Further evidence supporting the correlation between TEWL and IS data was found because, during tape stripping, the rate of TEWL increase corresponded to the rate of impedance decrease (for example, Subject A had the fastest TEWL increase and the steepest decrease in skin impedance, whereas Subject B had the slowest increase in TEWL and the slowest decrease in skin impedance).

After the tape stripping experiments were completed, the recovery of barrier function, as assessed by the return of TEWL and skin impedance to basal levels, was followed at 24-h intervals over a period of several days; the impedance spectra for Subject C are shown in Fig. 6 *a*. Using the normalization approach described above for Fig. 4, and choosing a specific low-frequency (10.12 rad s^{-1}) impedance, we made a comparison of the recovery of (normalized) basal impedance and TEWL levels for Subject C over a period of 5 days (Fig. 6 *b*). The IS data indicate that there was minimal recovery after a period of 24 h, with the

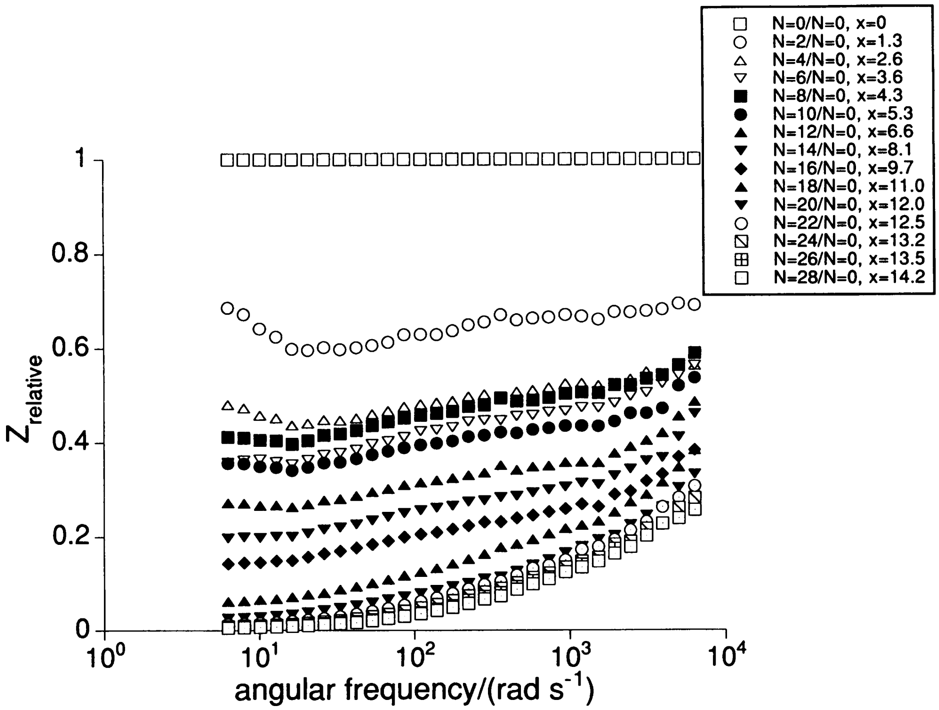

FIGURE 3 Skin impedance measured as a function of angular frequency at different SC depths during serial tape-stripping. The raw data are normalized by dividing the impedance at a given frequency by the impedance at that frequency when measured at the skin surface, $Z_{rel} = Z_{N=i}(\omega)/Z_{N=0}(\omega)$. This presentation of the data illustrates the differential effect of tape-stripping on the impedance across the frequency spectrum. Data from Subject B.

impedance response remaining almost constant across the frequency spectrum (Fig. 6 *a*). However, there was a noticeable change on the second day after tape stripping: the low-frequency impedance began to increase and TEWL showed a clear decrease (Fig. 6, *a* and *b*). By the third day, a significant change was observed with nearly complete recovery of the full magnitude of the impedance at low frequency. The TEWL measurements showed a similar trend, although recovery time was longer, 5–6 days generally being required before TEWL values returned to approximately basal levels.

DISCUSSION

The impedance of skin must, by definition, derive from the opposition to current flow, in the form of ion flux, under the influence of a potential gradient. The high tortuosity of the lipophilic pathway through the stratum corneum (Potts and Francoeur, 1990, 1991), which is a highly efficient barrier to diffusion, also contributes to the observed impedance. Our results demonstrated that, although compromising SC integrity was a sine qua non for reducing skin impedance and elevating TEWL, the removal of successive layers of the SC had different effects on these properties. Whereas removal of the uppermost layer of the SC caused the largest single change in skin impedance, it had little effect on TEWL—the latter was most affected by removal of the deeper layers. Although the SC regulates water loss to the external environment, small-angle x-ray diffraction studies have shown that it does require a minimal water content to maintain its structural integrity (Bouwstra et al., 1991). Therefore, once the upper layer of the SC has been removed, there is less opposition to ion flow. However, removal of the upper layer causes insufficient upheaval to significantly increase water loss. The deeper layers of the SC must be perturbed before a significant change in water flux can be detected by the evaporimeter. Successive layers of the SC show progressively greater cohesive properties and are removed more uniformly by the tape stripping procedure (King et al., 1979). At first sight this suggested that there was a gradation in water-regulating ability through the SC, with the innermost layers bearing the brunt of the responsibility for controlling water flux. However, were the data truly inconsistent with a homogeneous barrier to water flux?

Is the stratum corneum a Fickian membrane?

The flux (J) of water across the SC (i.e., the TEWL) can be written in terms of Fick's First Law of Diffusion (Fick, 1855):

$$J = \frac{(K \cdot \Delta C)\bar{D}}{H} = K_P \cdot \Delta C, \qquad (1)$$

where K is the SC-viable tissue partition coefficient of water (which may be expected to be ~0.06; Potts and Francoeur, 1991); $\bar{D}$ is the average apparent diffusivity of water in the SC of thickness, H (μm); and ΔC is the water concentration difference across the membrane (tissue concentration minus that in the atmosphere above the skin surface, i.e., ~55 M ≈ 1 g cm^{-3}). K_P (= $K\bar{D}/H$) is called the permeability coefficient of water across the SC.

During progressive removal of the SC by repeated tape stripping, TEWL begins to increase because of perturbation

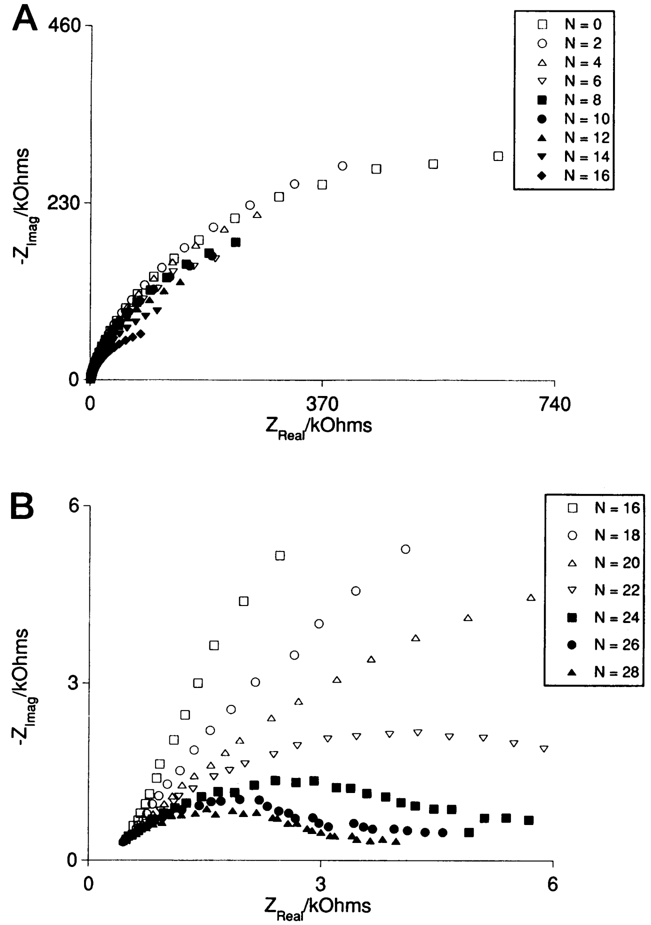

FIGURE 4 Effect of serial tape-stripping on the impedance locus. The complex plane impedance plot displays the variation of the imaginary (reactive) and real (resistive) components of the impedance (Z_{Imag}, versus Z_{Real}, respectively) (Jack et al., 1975). Note the large change in scale. Data from Subject B.

of the barrier. Mathematically, Eq. 1 must be modified to account for the gradual destruction of the barrier:

$$J_x = \frac{(K\cdot\Delta C)\bar{D}_x}{H-x} = K_P^x\cdot\Delta C, \tag{2}$$

where J_x is the TEWL value when x μm of SC has been removed by tape stripping and $\bar{D}_x$ is the average apparent diffusivity of water through the remaining $(H - x)$ μm of the SC; K_P^x is the corresponding modified permeability coefficient, i.e., $K_P^x = K\bar{D}_x/(H - x)$. Equation 2 can be linearized by inversion to give

$$\frac{1}{J_x} = \frac{H}{\gamma\bar{D}_x} - \frac{x}{\gamma\bar{D}_x}, \tag{3}$$

where $\gamma = K\Delta C$ and can reasonably be assumed to be constant. If the SC behaves as a homogeneous diffusion barrier to water (i.e., $\bar{D}_x$ is independent of x and equals $\bar{D}$), then a plot of $1/J_x$ versus x will be linear and the slope and intercept of the graph will yield explicit values for $\bar{D}$ and H (again, assuming that K is also independent of x and equals the value (0.06) determined earlier; Potts and Francoeur, 1991).

The experimental data for three subjects, plotted according to Eq. 3, are shown in Fig. 7. The results are highly linear, supporting the simple Fickian model. Derived values of $\bar{D}$ and H are given in Table 2, together with the water permeability coefficients deduced from the initial TEWL readings before tape stripping; the latter are within a factor of 2–3 of those reported previously in the literature (Scheuplein, 1965; Scheuplein and Blank, 1971; Astley and Levine, 1976; Akhter et al., 1984; Harrison et al., 1984; Bond and Barry, 1988). The derived values of H show that the tape stripping procedure achieves, or comes close to, removal of all of the SC (compare with the results in Table 1). It should be emphasized that the diffusivity values presented are apparent and are based upon the diffusion path length of water through the SC equaling the membrane's thickness. In other words, the tortuosity associated with, for example, an intercellular, lipid route of transport (as deduced by Potts and Francoeur, 1991, and others) is not accounted for by the analysis undertaken.

From the derived values of H, and the cumulative amount of SC removed as a function of tape stripping, the thickness of SC remaining $(H - x)$ after each tape strip was easily calculated. This then permits the manner in which the water concentration profile across the SC adjusts during sequential tape stripping to be deduced, provided that the establishment of the new steady state is achieved during the time that elapses between successive tape strips. Assuming that a maximum of 0.5 μm is removed per tape strip (see data in Table 1 for Subject B), the characteristic time (t^*) for water diffusion across the SC is a maximum, $t^* \approx (14.1 - 0.5)^2/2D \approx 200$ s (using the corresponding in Table 2), which is much less than the time between tape strips. It was therefore possible to construct relative water concentration profiles across the SC, as a function of progressive SC removal, for each subject (Fig. 8).

Implications for SC water permeability

Fig. 9 shows the variation of K_P^x $(= J_x/\Delta C)$ as a function of the thickness of the remaining SC $(H - x)$ during the tape stripping procedure. As membrane permeability is inversely proportional to its diffusional resistance (Crank, 1975), it follows that K_P^x increases slowly at first, but then quite dramatically once a significant fraction of the SC has been removed (i.e., as $H - x$ decreases). A further test of the homogeneity of the diffusional barrier can be made by plotting $\bar{D}_x$ as a function of $H - x$ using the values of K_P^x directly calculated from the TEWL measurements, i.e.,

$$\bar{D}_x = \frac{K_P^x(H-x)}{K}. \tag{4}$$

The results in Fig. 10 show that $\bar{D}_x$ is indeed independent of $H - x$, and that the values of $\bar{D}$ determined from the data

TABLE 1 Transepidermal water loss across the SC during serial tape stripping in three human subjects

Tape strip number	Subject A		Subject B		Subject C	
	TEWL*	Depth#	TEWL	Depth	TEWL	Depth
0	5.6	0.0	5.7	0.0	8.6	0.0
2	6.4	1.1	7.1	1.3	9.6	1.4
4	6.2	2.0	7.9	2.6	10.5	2.3
6	8.2	3.0	8.2	3.6	10.5	3.0
8	7.2	3.9	9.2	4.3	13.0	4.2
10	10.3	4.9	7.0	5.3	16.0	5.2
12	13.8	6.0	9.2	6.6	16.6	6.0
14	24.2	6.8	12.3	8.1	21.0	7.7
16	40.8	7.6	15.4	9.7	26.9	8.5
18	59.6	8.3	19.3	11.0	35.4	9.1
20	89.5	9.0	29.2	12.0	44.6	9.8
22	102.5	9.6	32.3	12.5	47.6	10.4
24			33.6	13.2		
26			41.3	13.8	58.4	11.3
28			42.3	14.1		

* TEWL measured in g m^{-2} h^{-1}.

\# Skin depth (μm) determined from the weight of stratum corneum removed by the tape strip (see text).

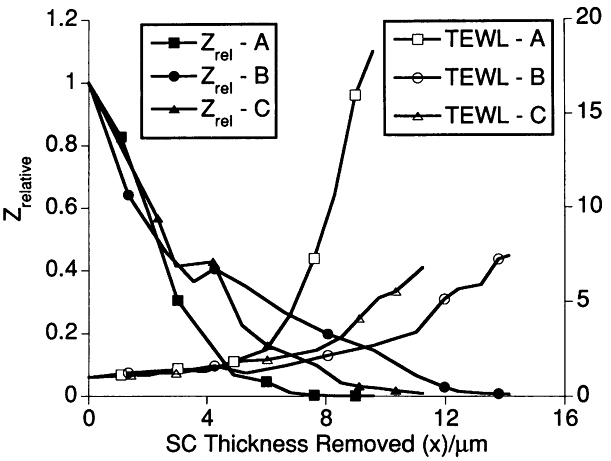

FIGURE 5 Variation of skin impedance (measured at a frequency of 10.12 rad s^{-1}) and TEWL as a function of SC thickness removed (x). $TEWL_{relative}$ is determined by dividing the TEWL measurement at a given depth by the basal TEWL value. $Z_{relative}$ is determined as described in the legend of Fig. 3. Data from Subjects A, B, and C.

expressed in this way are essentially identical to those given in Table 2.

Correlating skin impedance with skin hydration

As described above, the impedance is determined by the extent of opposition to ion transport and, because the latter is favored by an aqueous environment, skin impedance must be intimately related to the degree of skin hydration. Therefore, the SC impedance changes, as a function of tape stripping, were correlated with the changing water concentration in the remaining SC. As the boundary conditions for the internal and external water concentrations are fixed, and because the distance across which this concentration drop occurs decreases with each successive tape strip, the concentration gradient must increase. A corollary of the steepening of the water gradient is that, at a given distance from the skin surface, there must be an increase in the water

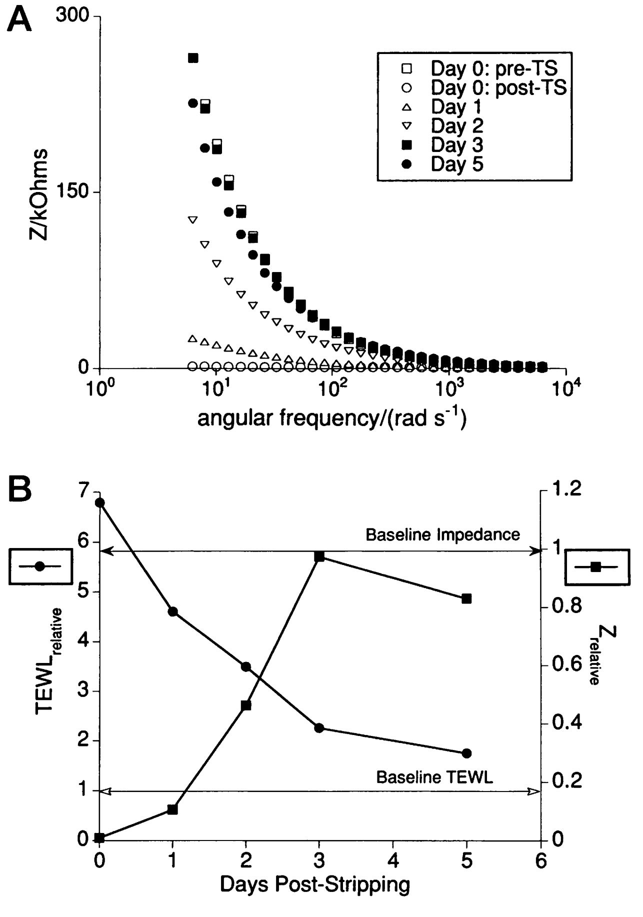

FIGURE 6 (a)Recovery of the skin impedance, and (b) recovery of $TEWL_{relative}$ and $Z_{relative}$ (ω = 10.12 rad s^{-1}) as a function of time after serial tape-stripping. Data from Subject C.

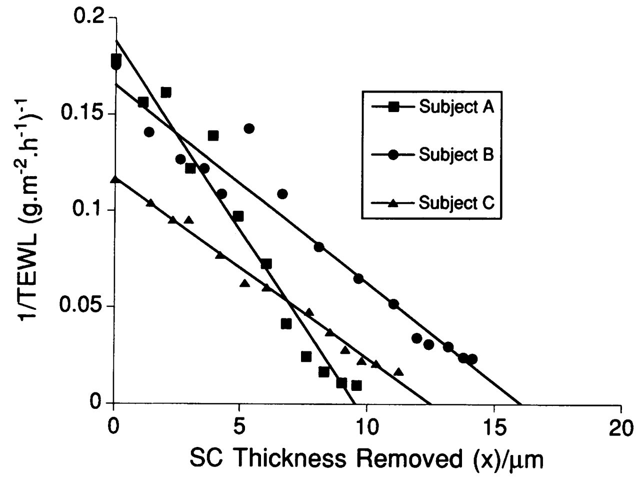

FIGURE 7 The dependence of 1/TEWL upon stratum corneum thickness. Highly linear fits were obtained for all three subjects. Subject A, $(\mathrm{TEWL})^{-1} = 0.188 - 0.0197x$ ($r^2 = 0.962$); Subject B, $(\mathrm{TEWL})^{-1} = 0.165 - 0.0103x$ ($r^2 = 0.949$); Subject C, $(\mathrm{TEWL})^{-1} = 0.117 - 0.00932x$ ($r^2 = 0.991$).

TABLE 2 Water diffusivities ($\bar{D}$), SC thicknesses (H), and SC permeability coeffcients of water (K_P), from TEWL data as a function of tape stripping

Subject	$10^9 \cdot \bar{D}$ (cm^2 s^{-1})*	H (μm)#	10^7 K_P (cm s^{-1})§
A	2.3	9.5	1.56
B	4.6	16.1	1.58
C	4.6	12.6	2.38
$\bar{x}$	3.83 ± 1.32	12.7 ± 3.3	1.84 ± 0.47

* Deduced from the slope of 1/TEWL versus SC thickness removed (x) (see Fig. 7), assuming $K = 0.06$ (Potts and Francoceur, 1991) and $\Delta C = 1$ g cm^{-3}.

\# Equal to the intercept on the ordinate axis of the plot of 1/TEWL versus SC thickness removed (x) (Fig. 7).

§ $K_P = \frac{K \cdot \bar{D}}{H} = \frac{J}{\Delta C}$ (Eq. 1).

concentration and therefore a decrease in skin impedance. Fig. 11 shows that the impedance decreased as the gradient of the relative water concentration increased (i.e., with sequential tape stripping) and tended to approach asymptotically a minimal value that presumably approximates to the impedance of the essentially aqueous tissue underlying the SC.

Correlation between skin impedance and skin permeability

Fig. 12 shows that the skin admittance ($Y = 1/Z$) is highly correlated with the SC water permeability K_P^x. The admittance is proportional to the water concentration, because an aqueous environment facilitates ion transport, which in turn is responsible for current flow. The permeability values represent the changing "velocity" of water molecules through the successively decreasing thickness of the SC. These progressively more freely moving molecules make ion transport more energetically favorable. Thus, as SC thickness is reduced and the "velocity" of water molecules increases, there is a corrresponding increase in ion concentration and the admittance rises accordingly.

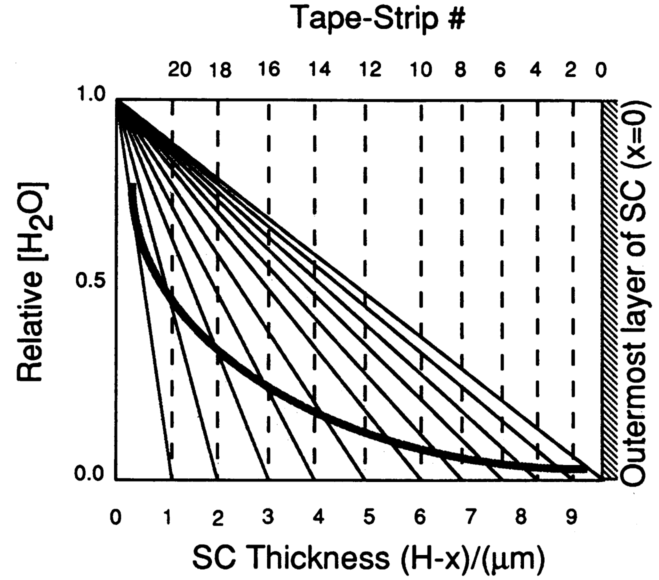

FIGURE 8 Water concentration profiles across the stratum corneum as a function of serial removal of the tissue by sequential tape-stripping. Data from Subject A.

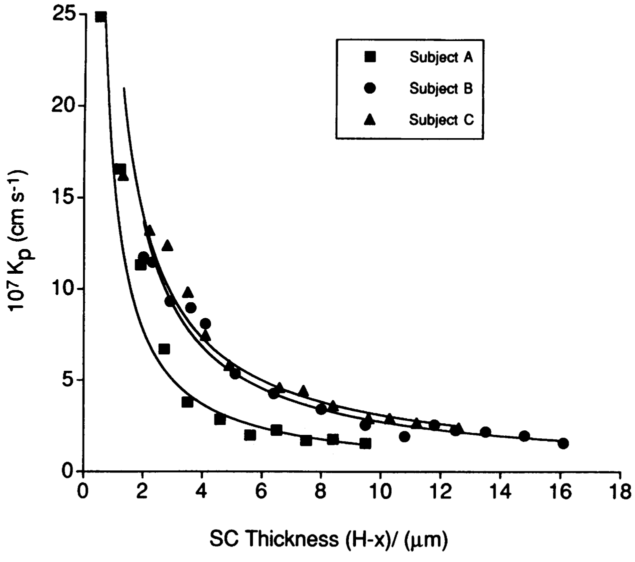

FIGURE 9 Dependence of K_P^x on the thickness of stratum corneum removed by sequential tape-stripping.

Recovery process

In terms of skin recovery, the impedance appears to recover faster than TEWL. The external "sheath" is formed first, and this re-creates the primary barrier to ion transport (Grubauer et al., 1989). Full functionality with respect to

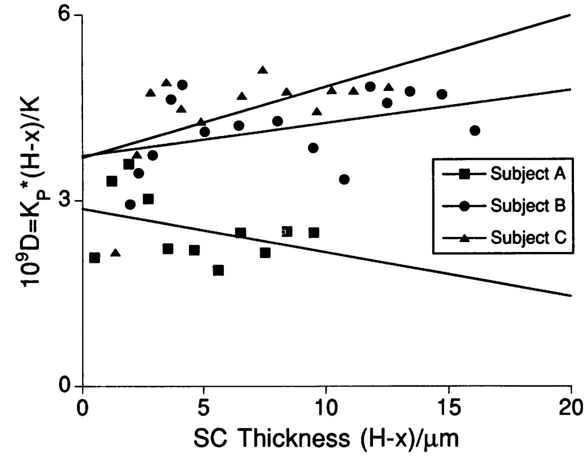

FIGURE 10 Dependence of water diffusivity ($\bar{D}$) across the stratum corneum as the thickness of tissue remaining ($H - x$) during sequential tape-stripping. Linear regression of the data indicates that $\bar{D}$ is independent of position. Subject A, (cm^2 s^{-1}) = 2.87 − 0.071($H - x$), (r^2 = 0.156); Subject B, (cm^2 s^{-1}) = 3.73 − 0.052($H - x$), (r^2 = 0.178); Subject C, (cm^2 s^{-1}) = 3.70 − 0.113($H - x$), (r^2 = 0.301).

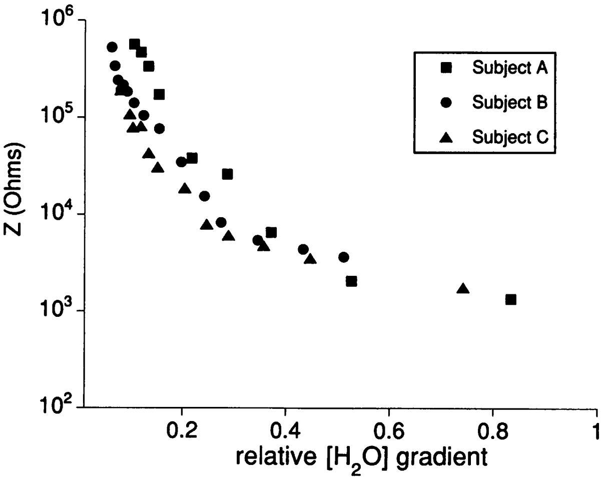

FIGURE 11 Relationship between SC impedance and the gradient of the relative water concentration profile across the tissue. $(H - x)^{-1}$ = (relative$[H^2O]/(H - x)$) ≡ relative$[H_2O]$gradient. Data from Subjects A, B, and C.

TEWL, however, requires additional, compact SC layers and takes a correspondingly longer period of time.

CONCLUSIONS

In summary, our results show that TEWL and IS are important complementary techniques for determining SC barrier function status and for the examination of water transport properties across the SC. The measurements are clearly related to key structures and to the principal function of the SC and are, as we have shown previously with respect to iontophoresis (Kalia et al., 1996), usefully distinct probes for different aspects of stratum corneum integrity. The SC consists of two very distinct and complicated domains: 1) the intercellular multilamellar arrangement of a complex mixture of lipids (primarily ceramides, fatty acids, and unesterified sterols) arranged into multiple phases (Ongpipattanakul et al., 1991; Gay et al., 1994), and 2) keratin-filled corneocytes, the envelopes of which interact closely with the surrounding lipid framework. The SC is also punctuated by the appendages (hair follicles and sweat glands), which add further structural complexity. In summary, therefore, it is clear that the SC comprises a unique membrane of diverse characteristics. And yet, the data presented here demonstrate that the SC, a structurally heterogeneous membrane, behaves as a homogeneous barrier to water transport in vivo. This may imply that the transport route remains homogeneous throughout—the overall pathway involves passage through successive SC layers, each layer making an equivalent contribution to overall barrier function. We have determined the in vivo diffusivity of water through the SC and obtained a value that is consistent with a simple physical interpretation of the data. Furthermore, the results show a strong correlation between skin hydration and skin impedance, a relationship that should be extremely useful for the optimization of formulations designed either to improve skin "health" or to facilitate drug delivery.

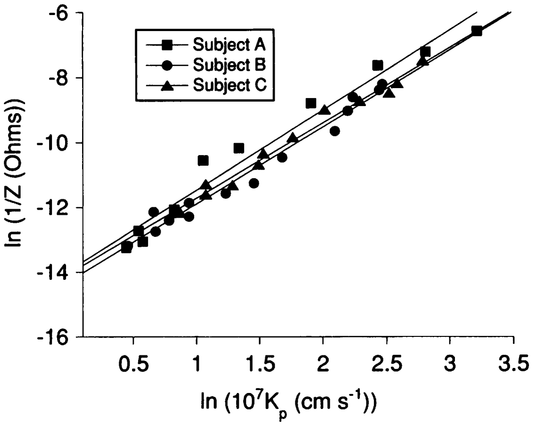

FIGURE 12 Relationship between skin admittance (1/Z) and water permeability across the stratum corneum. Highly linear correlations were obtained for all subjects: A (r^2 = 0.965); B (r^2 = 0.976); C (r^2 = 0.982).

Financial support was provided by the U.S. National Institutes of Health (HD-23010 and HD-27839), the U.S. Air Force Office of Scientific Research, and the U.S. Environmental Protection Agency. FP is particularly grateful to Prof. P. Agache (Centre de Recherche sur le Tégument, France), Hoffmann-LaRoche, and the Philippe Foundation.

REFERENCES

Akhter, S. A., S. L. Bennett, I. L. Waller, and B. W. Barry. 1984. An automated diffusion apparatus for studying skin penetration. *Int. J. Pharm.* 21:17–26.

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

Astley, J. P., and M. J. Levine. 1976. Effect of dimethyl sulfoxide on permeation of skin in vitro. *Pharm. Sci.* 65:210–216.

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

Bond, J. R., and B. W. Barry. 1988. Limitations of hairless mouse skin as a model for in vitro permeation through human skin: hydration damage. *J. Invest. Dermatol.* 90:486–489.

Bouwstra, J. A., G. S. Gooris, J. A. van der Spek, W. Bras. 1991. Structural investigations of human stratum corneum by small-angle X-ray scattering. *J. Invest. Dermatol.* 19:1005–1012.

Chapman, S. J., and A. Walsh. 1990. Desmosomes, corneosomes, and desquamation: an ultrastructural study of adult pig epidermis. *Arch. Dermatol. Res.* 282:304–310.

Chapman, S. J., A. Walsh, S. M. Jackson, and P. S. Friedmann. 1991. Lipids, proteins and corneocyte adhesion. *Arch. Dermatol. Res.* 283: 167–173.

Crank, J. 1975. The Mathematics of Diffusion, 2nd Ed. Clarendon Press, Oxford.

Downing, D. T. 1992. Lipid and protein structures in the permeability barrier of mammalian epidermis. *J. Lipid Res.* 33:301–313.

Fick, A. E. 1855. V. On liquid diffusion. *Philos. Magazine.* 10:30–39.

Frodin, T., and M. Skogh. 1984. Measurement of transepidermal water loss using an evaporimeter to follow the restitution of the barrier layer of human epidermis after stipping the straum corneum. *Acta Derm. Venereol. (Stock.).* 64:537–540.

Gay, C. L., R. H. Guy, G. M. Golden, and V. H. W. Mak. 1994. Characterization of low-temperature (ie, <65°C) lipid transitions in human stratum corneum. *J. Invest. Dermatol.* 103:233–239.

Golden, G. M., D. B. Guzek, A. H. Kennedy, J. E. McKie, and R. O. Potts. 1987. Stratum corneum lipid phase transitions and water barrier properties. *Biochemistry.* 26:2382–2388.

Grubauer, G., P. M. Elias, and K. R. Feingold. 1989. Transepidermal water loss: the signal for recovery of barrier structure and function. *J. Lipid Res.* 30:323–33.

Harrison, S. M., B. W. Barry, and P. H. Dugard. 1984. Effects of freezing on human skin permeability. *J. Pharm. Pharmacol.* 36:261–262.

Jack, J. J. B., D. Noble, and R. W. Tsien. 1975. Electric Current Flow in Excitable Cells. Oxford University Press, Oxford.

Kalia, Y. N., and R. H. Guy. 1995. Electrical characteristics of human skin in vivo. *Pharm. Res.* 12:1605–1613.

Kalia, Y. N., L. B. Nonato, and R. H. Guy. 1996. Correlation between impedance spectroscopy and transepidermal water loss: non-invasive probes of human skin permeability in vivo. *In* Prediction of Percutaneous Penetration, Vol. 4. K. R. Brain, V. J. James, and K. A. Walters, editors. STS Publishing, Cardiff. 38–41.

King, C. S., S. P. Barton, S. Nicholls, and R. Marks. 1979. The change in properties of the stratum corneum as a function of depth. *Br. J. Dermatol.* 100:165–172.

Kontturi, K., and L. Murtomäki. 1994. Impedance spectroscopy of human skin. A refined model. *Pharm. Res.* 11:1355–1357.

Liron, Z, H. J. Clewell, and J. N. McDougal. 1994. Kinetics of water vapor sorption in porcine stratum corneum. *J. Pharm. Sci.* 83:692–698.

McAdams, E. T., and J. Jossinet. 1991. The importance of electrode-skin impedance in high resolution electrocardiography. *Automedica.* 13: 187–208.

Obata, M., and H. Tagami. 1989. Electrical detrmination of water content and concentration profile in a simulation model of in vivo stratum corneum. *J. Invest. Dermatol.* 92:854–859.

Ongpipattanakul, B., R. R. Burnette, R. O. Potts, and M. L. Francoeur. 1991. Evidence that oleic acid exists in a separate phase within stratum corneum lipids. *Pharm. Res.* 8:350–354.

Pinkus, H. 1951. Examination of the epidermis by the strip method of removing horny layers. *J. Invest. Dermatol.* 16:383–386.

Pirot, F., F. Panisset, P. Agache, Y. N. Kalia, and P. Humbert. 1995. Comparison of four noninvasive quantitative probes of skin irritation induced by sodium lauryl sulphate. *Eur. J. Dermatol.* 5:709–714.

Potts, R. O., and M. L. Francoeur. 1990. Lipid biophysics of water loss through the skin. *Proc. Natl. Acad. Sci. USA.* 87:3871–3873.

Potts, R. O., and M. L. Francoeur. 1991. The influence of stratum corneum morphology on water permeability. *J. Invest. Dermatol.* 96:495–499.

Scheuplein, R. J. 1965. Mechanism of percutaneous absorption. I. routes of penetration and the influence of solubility. *J. Invest. Dermatol.* 45: 334–346.

Scheuplein, R. J., and I. H. Blank. 1971. Permeability of the skin. *Physiol. Rev.* 51:702–747.

Tregear, R. T. 1966. Physical Functions of Skin. Academic Press, New York.

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

Wertz, P. W., D. C. Swartzendruber, W. Abraham, K. Madison, and D. T. Downing. 1987. Essential fatty acids and epidermal integrity. *Arch. Dermatol.* 123:1381–1384.

Williams, M. L., and P. M. Elias. 1987. The extracellular matrix of the stratum corneum: role of lipids in normal and pathological functions. *CRC Crit. Rev. Ther. Drug Carrier Syst.* 3:95–112.

Wolf, J. 1939. Die innere Struktur der Zelles des Stratum Desquamans der mensclichen Epidermis. *Z. Zellforsch. Mikrosk. Anat.* 46:270–202.

Yamamoto, T., and Y. Yamamoto. 1976. Electrical properties of the epidermal stratum corneum. *Med. Biol. Eng.* 14:151–158.