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Report

Assessment of Skin Barrier Function Using Transepidermal Water Loss: Effect of Age

Kathleen V. Roskos[1] and Richard H. Guy[1,2]

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To probe age-related changes in skin barrier function, transepidermal water loss (TEWL) rates have been measured in "young" (19–42 years) and "old" (69–85 years) subjects. TEWL was determined at ventral forearm skin sites, which had been occluded for 24 hr with polypropylene chambers. Baseline TEWL rates (J_∞), which showed no dependence on age, were measured for each subject before and after the experiment. Following removal of the occlusive chamber, TEWL was monitored continuously from $t = 0.5$ min until its return to the baseline (preocclusion) level, which was typically in the range of 2–7 g/m^2/hr. Initial TEWL rates (mean ± SD) were found to differ significantly between young (28.6 ± 7.5 g/m^2/hr; $n = 26$) and old (36.9 ± 10.5 g/m^2/hr; $n = 18$) subjects ($P < 0.01$). Relaxation of TEWL to J_∞ was significantly slower in the aged cohort, such that the characteristic time for diffusion of water in the stratum corneum was estimated to be (mean ± SD) 176 ± 59 min for the young subjects, compared to 360 ± 76 min for the old ($P < 0.001$.). Thus, the initial TEWL value following removal of occlusion is significantly greater, and the excessive stratum corneum hydration produced by occlusion is dissipated more slowly, in old skin than in young. A hypothesis to explain the slower relaxation of perturbed TEWL in old skin is proposed.

KEY WORDS: transepidermal water loss; age; barrier function; stratum corneum occlusion.

INTRODUCTION

Water reaches the surface of the skin actively via the sweat ducts and by passive diffusion across the intact epidermis. Measurement of the latter process, transepidermal water loss (TEWL), is a sensitive evaluation of the integrity of the stratum corneum (SC). The SC is the thin (15 to 20-μm) coherent membrane of keratinized epithelial cells which form the outermost layer of the epidermis. The SC consists of a closely packed latticework of polyhedral cells arranged as interlocked vertical columns (1). The intercellular regions of the SC contain predominately lipoidal material in a multilamellar bilayer arrangement (2). A principal function of the SC is to prevent excessive water loss from the body and to maintain homeostasis.

Previous studies have determined evaporative water loss across the skin as a function of increasing chronologic age. For example, measurements of TEWL have been used to assess adequacy of barrier function in premature infants (<30 gestational weeks) relative to that in full-term infants (3): it was found that water evaporation rate from the skin is higher in preterm infants than in term infants. At the opposite end of the age spectrum, i.e., from adulthood through old age, baseline TEWL does not appear to change with increasing age (4–6).

The objective of this study was to reexamine the effect of increasing age on barrier function integrity as assessed by TEWL. Initial measurements confirmed the previously published observation that baseline TEWL does not change significantly with increasing age. This led us to examine the water barrier under "stressed" conditions: the skin was first occluded, thereby preventing TEWL, and then the recovery of the perturbed tissue to pretreatment levels was monitored. It was our hypothesis that this challenge to the skin would amplify subtle barrier function changes that occur with increasing age. Evidence that occlusion would enhance the "signal-to-noise" ratio associated with TEWL measurements was indicated by previously published experiments, which revealed significant reversible increases in TEWL due to occlusion (7). In the experiments described below, occlusion-induced hydration changes in the SC were monitored by the relaxation of TEWL to baseline (preocclusion) levels.

MATERIALS AND METHODS

Transepidermal water loss measurements were performed with a commercially available, unventilated evaporimeter (EPIC Servomed AB, Stockholm, Sweden). The Servomed EPIC is designed for the quantitative determination of water evaporation from or to surfaces in contact with the atmosphere. The evaporimeter probe has been described elsewhere in detail (8–11). Briefly, the instrument is capable of measuring relative air humidity (%), water vapor partial

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[1] Departments of Pharmaceutical Chemistry and Pharmacy, University of California, San Francisco, San Francisco, California 94143.

[2] To whom correspondence should be addressed at School of Pharmacy, University of California, San Francisco, California 94143-0446.

pressure (mm Hg), and evaporation rate (g/m^2/hr) (to a lower limit of 0.1 g/m^2/hr). The evaporimeter probe contains two sensors (coated with an organic polymer dielectically sensitive to humidity) vertically stacked at a known distance from the skin surface. Each sensor is coupled to a fast thermistor and both are enclosed in a polytetrafluoroethylene capsule, cylindrically shaped and open at both ends. The probe is held against the surface of the skin, orienting the sensors parallel to the skin surface and perpendicular to the vapor pressure gradient. Investigations of transepidermal water loss can be performed, therefore, with minimal influence of ambient conditions on the microclimate at the surface of the skin. The TEWL reading from the probe stabilizes within approximately 15–30 sec.

Volunteers were caucasian males and females divided into two groups; young (19–42 years) (n = 13) and old (69–85 years) (n = 9). Volunteers had no history of dermatological disease. All measurements were made on the ventral forearm (bilateral sites) in a draft-free environment; relative humidity was 30–50% and ambient temperature 23 ± 2°C. Since large experimental variation due to such factors as ambient temperature, relative humidity and rate of perspiration can invalidate TEWL results, well-controlled experimental conditions were maintained. The skin temperature during measurement, on any given individual, was <33°C at a room temperature of <25°C. All TEWL measurements were normalized to a 30°C skin temperature as previously described (12).

Occlusion was achieved using a 2-cm-diameter, polypropylene Hilltop chamber (HTC) (Hilltop Research, Inc., Cleveland, Ohio). Preliminary investigations sought to obtain an occlusion time for complete SC hydration. Occlusion times of 1, 2, 6, 12, 24, 36, and 48 hr were examined. In light of these experiments (discussed below), we chose to occlude the skin for 24 hr. This treatment optimized volunteer compliance with full SC hydration in the absence of maceration.

RESULTS AND CALCULATIONS

We have confirmed the observations of others (4–6) that baseline TEWL does not change with increasing age (Fig. 1). It is unlikely, therefore, that baseline TEWL can be used to differentiate barrier function with increasing age. Hence, to probe whether pertubation of SC hydration reveals age-associated changes in barrier function, we measured TEWL rates following simple occlusion.

The preliminary experiments revealed that for shorter occlusive periods (1 and 2 hr), the SC did not always achieve full hydration, i.e., the initial TEWL values following removal of the HTC were significantly lower than the corresponding values after 6, 12, or 24 hr of occlusion. TEWL following 36 and 48 hr of occlusion occasionally exhibited unexpected behavior in that water loss remained elevated at a constant level for a protracted period (ca. 4 min) before commencing the typical relaxation to baseline values. The reason for this extended plateau is not known or readily discernible but may be associated with an artifact resulting from desquamated cells lying on the skin surface. Results on any individual were essentially identical following occlusion times of 6, 12, and 24 hr suggesting that the SC had reached a true equilibrium.

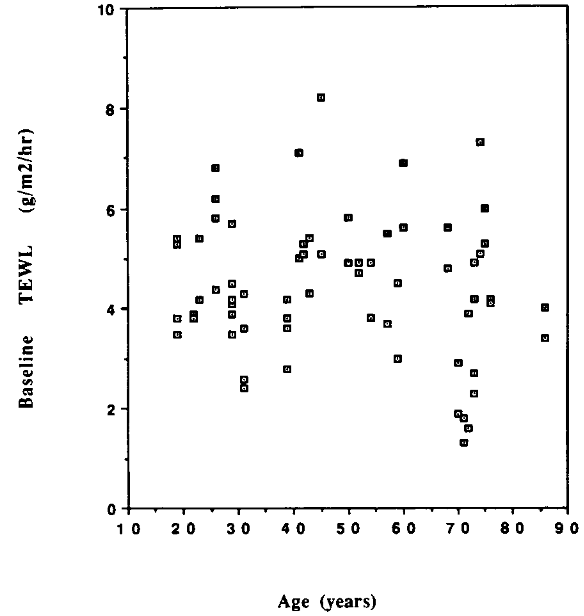

Fig. 1. Baseline TEWL readings from 33 subjects in the age range 19–85 years. Measurements were made on the ventral forearm.

Occlusion prevents the normal passive diffusion of water from the skin into the atmosphere. As a result, the water content of the stratum corneum rises, until equilibrium with the body occurs. Upon removal of the occlusion, TEWL is elevated above its baseline value, but then returns to this level as the linear concentration gradient across the SC is reestablished. Data from 26-year-old and 85-year-old individuals, following 24 hr of occlusion, illustrate this decay of water evaporation rate immediately following removal of the HTC (Fig. 2). Note the extended time scale for the older individual, illustrating the relative slowness of the relaxation process in this subject.

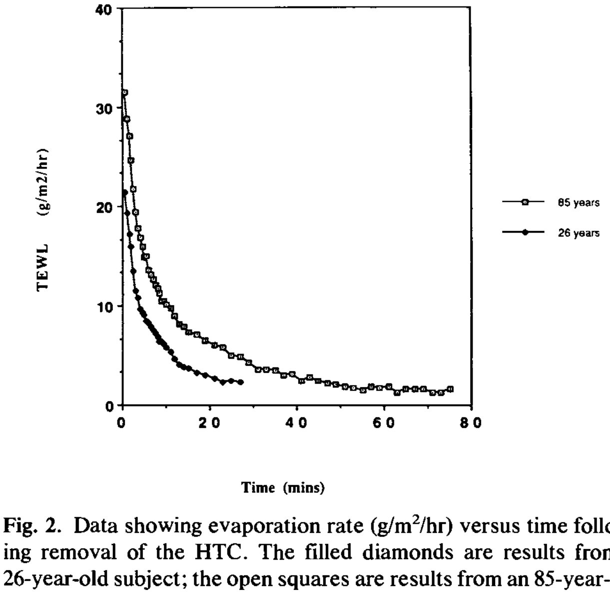

Fig. 2. Data showing evaporation rate (g/m^2/hr) versus time following removal of the HTC. The filled diamonds are results from a 26-year-old subject; the open squares are results from an 85-year-old subject. The occlusion time was 24 hr. Note the extended time scale for the older individual, illustrating the relative slowness of the "relaxation" process in this subject.

Schematically, this experiment produces changes in the water activity gradient across the stratum corneum shown in Fig. 3. Following removal of occlusion, the step-function in water activity collapses to the normal linear gradient. This relaxation process can be analyzed using standard diffusion theory (13), provided that a number of assumptions, implicit in the treatment of this non-steady-state diffusion problem by simple Fickian principles, are recognized.

(1) The SC is an isotropic membrane of constant thickness.
(2) The diffusion coefficient (D) of water across the SC is both distance and concentration independent.
(3) The temperature of the membrane is constant across its width and throughout the measurement.
(4) The atmosphere above the skin (at the skin–air interface) is a perfect sink for water following removal of the occlusive barrier.

To interpret the relaxation of TEWL rate (J) to its baseline value (J_∞), Fick's Second Law of Diffusion for water in the SC membrane must be solved:

$$\frac{\partial C}{\partial t} = D\left(\frac{\partial^2 C}{\partial x^2}\right) \tag{1}$$

with the boundary conditions

(i) $a_{H_2O} = 1, \quad 0 < x < l \text{ at } t = 0$
(ii) $a_{H_2O} = 1, \quad x = 0 \text{ for t} > 0$
(iii) $a_{H_2O} = 0, x = l \text{ for t} > 0$

where a_{H_2O} is the water activity in the SC. The mathematics permit one to evaluate the flux of water (J) exiting the SC (at $x = l$) as a function of time (t) (i.e., the quantity, TEWL, detected by the evaporimeter). The result (13), which is acceptable for all but the shortest times (i.e., once a_{H_2O} has decreased by about 30% or more), is

$$\frac{J}{J_\infty} = 1 + 2\exp\left(\frac{-D\pi^2 t}{l^2}\right) \tag{2}$$

where J_∞ is the average "control" (baseline) value measured for each subject at the beginning and end of each experiment. This function is plotted in Fig. 4 (13). The decay curve of J/J_∞ can therefore be used to find the characteristic transport time (l^2/D, where l = diffusional path length) for water diffusion across the SC. If $t = l^2/6D$ [the classic "lag time" (t_{lag})] in Eq. (2), then $J/J_\infty \approx 1.39$. Thus, by measuring J/J_∞ directly with the evaporimeter, t_{lag} and, hence, l^2/D can be easily obtained. The decay of J is monitored when occlusion is terminated and J/J_∞ is then calculated as a function of time. Table I shows that the flux at 30 sec postocclusion is greater for the old subjects than for the younger individuals. Additionally, the relaxation to control levels is significantly slower for the older group compared to the younger group. The $J_{15'}/J_\infty$ ratio and the characteristic diffusion time are significantly lower in the young individuals.

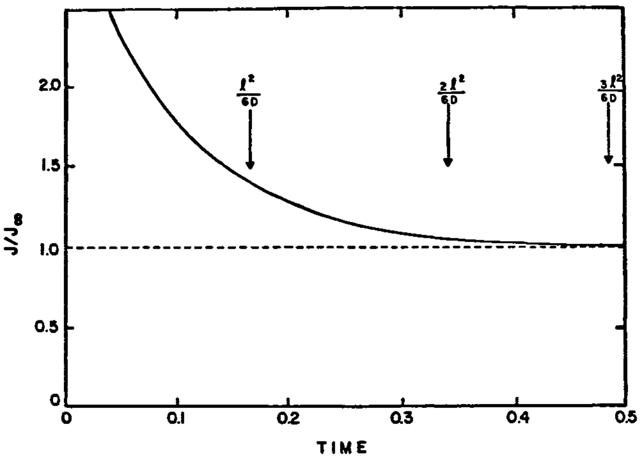

Fig. 4. Graphical representation of the approximate solution to Fick's Second Law (with appropriate boundary conditions) reveals that, for all but the shortest times, the decay of J/J_∞ with time can be used to find the lag time and characteristic transport ratio for the system.

An alternative, empirical, analysis of the TEWL data leads to similar conclusions. The TEWL relaxation measurements postocclusion are adequately fitted by a simple biexponential function:

$$(J\text{-}J_\infty) = A\exp(-\alpha t) + B\exp(-\beta t) \tag{3}$$

where J is the TEWL flux at time t postocclusion (units = g/m^2/hr); A and B are constants (units = g/m^2/hr) and alpha and beta are first-order decay constants (units = reciprocal time). A biexponential function was found to fit the TEWL data significantly better than a simple exponential (14); however, inclusion of additional exponential terms does not cause further improvement in data fit. While the approach is empirical, the rate constants obtained should be inversely related to the characteristic diffusion time (l^2/D) and can be used for comparative purposes between young and old subject groups (Table II).

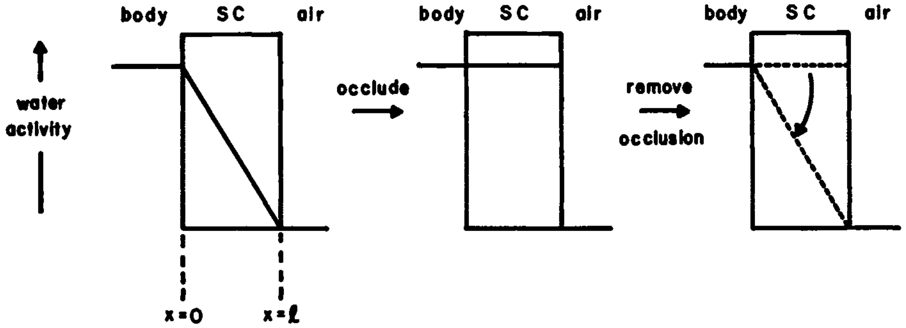

Fig. 3. Schematic representation of the occlusion-induced changes in water activity gradient across the stratum corneum.

Table I. TEWL Relaxation Data, Characteristic Diffusion Times Following 24 Hours of Occlusion (Mean ± SD)[a]

	Young (n = 26)	Old (n = 18)	P value
$J_{30''}$ (g/m²/hr)	29 ± 7.5	37 ± 11	0.004
$J_{15'}/J_\infty$	2.2 ± 0.5	3.6 ± 0.9	<0.001
l^2/D (min)	175 ± 59 (n = 16)	360 ± 76 (n = 14)	<0.001

[a] $J_{30''}$ = TEWL rate at t = 30 sec postocclusion. $J_{15'}/J_\infty$ = the ratio of TEWL rate at t = 15 min postocclusion to TEWL baseline rate (J_∞). l^2/D = the characteristic time for water diffusion across the SC.

Data analysis of the TEWL results assumes that vapor-phase diffusion of water from the skin surface to the relative humidity sensors in the evaporimeter probe is much faster than water diffusion through the SC, i.e., the resistance to water transport in the air layer above the skin is negligible. To verify the validity of this approximation, both continuous and discontinuous TEWL measurements were made and were found to be indistinguishable. Removal and replacement of the probe disturbs the air layer over the skin surface. If the water concentration gradient can be rapidly reestablished, then discontinuous values will overlap the continuously acquired values. The results (Fig. 5) demonstrate this to be the case.

DISCUSSION

As noted by ourselves and others, the ability of the SC to retard evaporative water loss does not change significantly with age. This result is perhaps not surprising. Extensive morphological data (15,16) give no evidence of a change in SC cell number or histologic appearance with increasing age. Additionally, as an individual ages, the SC maintains its physiologic function of preventing dehydration of the organism.

However, following a deliberate pertubation of the system, the water activity gradient across the SC (produced by occlusion) is dissipated more efficiently by the young group than by the old group. Specifically, we have observed an increase in the characteristic time for water diffusion through the older SC and a decrease in the kinetics which describe the relaxation of TEWL to baseline levels following occlusion. Additionally, the initial value of $J_{30''}$ is significantly greater in the old group than in the young.

The explanation of these observations is not intuitively obvious. Histologically, the SC appears unaltered with age.

Table II. Biexponential Analysis of TEWL Data (Mean ± SD)

	Young (n = 26)	Old (n = 18)	P value
A (g/m²/hr)	24 ± 10	30 ± 12	0.05
α (min^{-1})	0.47 ± 0.19	0.30 ± 0.16	0.01
B (g/m²/hr)	11 ± 3.9	13 ± 4.5	0.24
β (min^{-1})	0.05 ± 0.02	0.03 ± 0.02	0.01

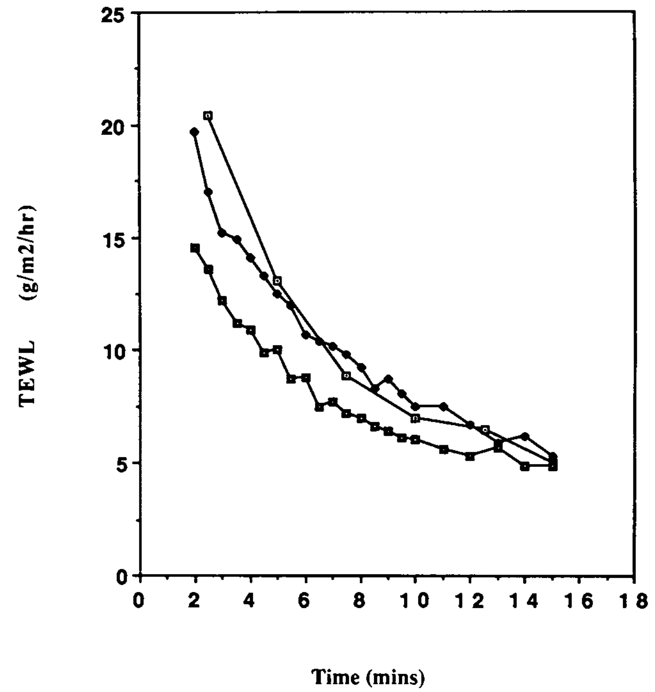

Fig. 5. Evaporation rate (g/m²/hr) versus time from a young subject (28 years) following occlusion for 12 hr. The filled diamonds and filled squares are readings taken continuously on two separate occasions. The open squares are measurements made discontinuously; between readings the evaporimeter was removed from the skin. The results also serve to illustrate the typical *intra*subject variability observed in TEWL observations.

Yet subtle changes appear to have taken place. It is known that the water content of older SC is lower (17,18). The dry, rough appearance of older skin implies a lower affinity for water. Consequently, the magnitude of the pertubation may account for the differences seen. From the baseline data, we know that the SC retains its ability to retard normal water loss over the entire age spectrum. In other words, while the total amount of water in the SC membrane may be different, the equilibrium TEWL rate is apparently unaltered with age. When the tissue is occluded, less water may be required by the younger tissue to achieve the fully hydrated equilibrium state. Conversely, the older tissue, starting at a lower water content, requires more water to achieve the new hydration equilibrium. Once the occlusion is removed, the SC from the older subjects may have more water to be eliminated (as reflected by a greater $J_{30''}$ value) and the relaxation to baseline may take longer due to the greater amount of water in the membrane. However, the kinetics of this relaxation process are significantly slower in the old subjects.

It is also possible that occlusion of the SC of old subjects produces a hydration-induced structural alteration in intercellular lipid organization. On subsequent dehydration, therefore, reorganization of this structural modification could be manifested in slower relaxation kinetics.

It is also conceivable that the differential relaxation behavior of TEWL in young and old subjects has origins, at least in part, in the keratinized components of the SC. Experiments probing SC structural components, at a microscopic level, will be required to understand the mechanism which accounts for the distinctive differences in observed TEWL relaxation behavior.

Finally, the validity of the assumptions, implicit in modeling TEWL observations by Fick's Second Law of Diffusion, should be reviewed. In reality, each assumption is imperfect. The SC is not isotropic; it is known that the integrity of the SC is weaker at the surface than at the viable epidermis–SC boundary. Occlusion induces hydration and swelling of the SC; hence, SC thickness probably decreases with time following removal of occlusion. The value of D is almost certainly dependent upon the degree of hydration and, as a result, will also be a function of SC depth. The final assumption is the most reasonable: the diffusion coefficient of water (D) in the vapor phase is several orders of magnitude faster than in the SC. A stagnant layer would need to be of very large dimension, therefore, to exercise a significant effect. Nevertheless, while the imperfection of the diffusional analysis is clear, the disparity in experimental results between old and young subjects is unequivocal. The model used serves a useful function, therefore, in that it quantifies the age-dependent differences in terms of familiar parameters. Further work will be necessary to refine the analysis in terms of a more physically precise description.

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REFERENCES

1. G. F. Odland. In L. A. Goldsmith (ed.), *Biochemistry and Physiology of the Skin,* Oxford University Press, New York, 1983, pp. 3–63.
2. P. M. Elias. *Int. J. Dermatol.* 20:1–19 (1981).
3. K. Hammarlund and G. Sedin. *Acta Paediatr. Scand.* 68:795–801 (1979).
4. K. A. Grice and F. R. Bettley. *Br. Med. J.* 4:195–198 (1967).
5. A. M. Kligman. *J. Invest. Dermatol.* 73:39–46 (1979).
6. J. L. Leveque, P. Corcuff, J. De Rigal, and P. Agache. *Int. J. Derm.* 23:322–329 (1984).
7. R. Aly, C. Shirley, B. Cunico, and H. I. Maibach. *J. Invest. Dermatol.* 71:378–381 (1978).
8. G. E. Nilsson and P. A. Oberg. 3rd Nordic Meeting on Medical and Biological Engineering, Tampere, 53, 1975, pp. 53.1–53.3.
9. G. E. Nilsson. *Med. Biol. Eng. Comput.* 15:209–218 (1977).
10. R. C. Scott, G. J. A. Oliver, P. H. Dugard, and H. J. Singh. *Arch. Dermatol. Res.* 274:57–64 (1982).
11. D. R. Wilson and H. I. Maibach. In H. I. Maibach and E. K. Boisits, (eds), *Neonatal Skin, Structure and Function,* Marcel Dekker, New York, 1982, pp. 83–100.
12. C. G. T. Mathias, D. M. Wilson, and H. I. Maibach. *J. Invest. Dermatol.* 77:219–220 (1981).
13. R. W. Baker and H. K. Lonsdale. In A. C. Tanquary and R. E. Lacey (eds.), *Controlled Release of Biologically Active Agents,* Plenum Press, New York, 1974, pp. 15–71.
14. *JANA Exponential Curve Stripping,* Statistical Consultants, Inc., Lexington, Ky., 1987.
15. R. M. Lavker. *J. Invest. Dermatol.* 73:59–66 (1979).
16. R. M. Lavker, P. Zheng, and G. Dong. In B. A. Gilchrest (ed.), *Dermatologic Clinics: The Aging Skin,* W.B. Saunders, Philadelphia, 1986, pp. 379–389.
17. R. O. Potts, E. M. Buras, and D. A. Chrisman. *J. Invest. Dermatol.* 82:97–100 (1984).
18. R. O. Potts and E. M. Buras. *J. Soc. Cosmet. Chem.* 36:169–176 (1985).