Mobility of Water in Human Stratum Corneum

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Received 15 July 2002; revised 29 January 2003; accepted 9 May 2003

ABSTRACT: At low water activities, stratum corneum (SC) water sorption resembles that in other keratinized tissues (i.e., wool and horn), whereas at high water activities, it resembles that in polymeric hydrogels. We propose that the concentration-dependent water diffusivity observed in these other systems applies to the corneocyte phase of the SC. An increase in SC hydration leads to increased water diffusivity in the corneocytes, in accordance with the predictions of both effective diffusion and free volume theories. Thus, theoretical results on effective diffusivity in a composite medium with random fiber obstacles and a free volume theory for water diffusivity in hydrogels (calibrated using data from wool and horn) have been applied to human SC water sorption data to estimate and establish theoretical limits on water diffusivity in corneocytes as a function of water activity. These results are used in conjunction with steady-state water permeability data to estimate the water permeability of both corneocyte and lipid phases of the SC under hydrated and partially hydrated conditions. The results of the analysis, when combined with previous spectroscopic analyses, strongly suggest that the lipids provide most of the SC water barrier in either case; thus, the diffusion pathway for water is primarily transcellular. J Pharm Sci 92:2326–2340, 2003

Keywords: permeability; percutaneous; transcellular transport; hydration; mobility, diffusion

INTRODUCTION

A concentration-dependent diffusivity for water in stratum corneum (SC) has been postulated by many investigators.[1–6] Various explanations have been offered. Blank and co-workers,[1] for example, thought that the increase in diffusivity at high hydration levels was a consequence of swelling of the corneocytes, whereas Potts and Francoeur[6] argued that it was related to hydration of the headgroups of intercellular lipids. Stockdale's analysis[5] suggested a tremendous increase in diffusivity as the SC approaches full hydration, although the mechanism was not specified. The following analysis provides a basis for Stockdale's observation in terms of increased diffusivity of water in the corneocyte phase. Overall water transport within the tissue, however, is determined by diffusion through both corneocyte and lipid phases, so it does not increase as rapidly as implied by Stockdale's analysis. The present analysis provides plausible upper and lower bounds for corneocyte water permeability, allowing its estimation to within a factor of ten for physiological values of SC hydration.

THEORY

Water Sorption Isotherm

Equilibrium water sorption in human SC was characterized in an accompanying paper.[7] Three isotherm models were shown to satisfactorily describe the sorption profiles obtained *in vitro*.

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Journal of Pharmaceutical Sciences, Vol. 92, 2326–2340 (2003)

We focus here on the Frenkel–Halsey-Hill (FHH) model, which has slight advantages versus alternative descriptions in terms of predictive power, but more important advantages as a conceptual model, due to its ability to describe long-range interactions. The equation is[7–9]

$$(v/v_m)^n = -\tilde{A}/\ln(a_w/a_0) \quad (1)$$

In this equation, a_w is water activity, v (the adsorption "volume") is the mass of water adsorbed expressed as g H_2O/g dry tissue, v_m is the mass of an adsorbed monolayer, and a_0, n, and $\tilde{A}$ are constants. The values of these parameters for human SC were $a_0 = 1.010$, $n = 1.07$, $\tilde{A} = 1.28$ when v_m was arbitrarily chosen to be equal to the Brunauer–Emmett–Teller (BET) value of 0.0386 g H_2O/g dry tissue.[7] The parameter a_0 in eq. 1 represents a minor modification to the original FHH equation, for which $a_0 = 1$.[9] This modification is required to limit the calculated amount of swelling when the tissue is immersed in pure water. In other words, choosing $a_0 > 1$ causes the value of v to remain finite when $a_w = 1$.

Water Diffusivity

The diffusivity, D, of sorbed water in a polymer system is, in general, a function of its concentration. For a planar membrane in which diffusion occurs in the z direction, Barrie[10] gives

$$J = -bRT\left(\frac{\partial \ln a_w}{\partial \ln C}\right)\frac{\partial C}{\partial z} = -D(C)\frac{\partial C}{\partial z} \quad (2)$$

where b is the intrinsic mobility of the sorbed water and C is its concentration. The quantity bRT is equivalent to the unadjusted diffusion coefficient D_0 that appears in the thermodynamic extension of Fick's Law,[11]

$$J = -\frac{D_0 C}{RT}\frac{\partial \mu}{\partial z} \quad (3)$$

in which $\mu = \mu^0 + RT \ln a$ is the chemical potential and $a = \gamma C$ is the activity (for water, $a = a_w$). This expression replaces Fick's Law for systems in which γ varies continuously with z or C.[11] Thus, the thermodynamically adjusted diffusivity of water in the membrane is

$$D(C) = D_0\left(\frac{\partial \ln a_w}{\partial \ln C}\right) \quad (4)$$

Equations 2–4 are to be used with concentrations *within the membrane*, not bulk concentrations in solutions at local equilibrium with the membrane at its boundaries. For clarification, please refer to eqs. 16–19 (vide infra). Our goal is to develop relationships for D_0 and $D(C)$ for water within the corneocyte phase of the SC. The expression for $D(C)$ will have two components, corresponding to the two factors in eq. 4, D_0 and $(\partial \ln a_w/\partial \ln C)$, both of which are concentration dependent. In a later section, we will use this relationship to estimate corneocyte water permeability under conditions of full and partial hydration.

It will prove convenient to define water concentration in the corneocyte, C, in terms of its volume fraction, φ_1. If C is expressed as g H_2O/cm^3 tissue and φ_1 as mL H_2O/mL of tissue, these quantities are numerically equivalent. The remainder of the corneocyte is primarily keratin, with a volume fraction of φ_2. Thus, $\varphi_1 + \varphi_2 = 1$.

Thermodynamic Correction Factor

The mass of adsorbed water per gram of tissue v is related to φ_1 by the expression[7]

$$v = \frac{\rho_1 \varphi_1}{\rho_{mem}(1 - \varphi_1)} \quad (5)$$

in which ρ_{mem} is the dry density of the tissue (1.3 g/cm^3 for SC)[1] and ρ_1 is the density of water (1.0 g/cm^3). Using this relationship and $C = \varphi_1$, differentiation of eq. 1 yields

$$\left(\frac{\partial \ln a_w}{\partial \ln C}\right) = \left(\frac{\partial \ln a_w}{\partial \ln v}\right)\left(\frac{\partial \ln v}{\partial \ln \varphi_1}\right) = \left(n \ln\frac{a_0}{a_w}\right)\left(\frac{1}{1 - \varphi_1}\right) \quad (6)$$

Estimation of D_0

An early attempt to estimate the unadjusted water diffusivity D_0 in a keratin–water system was made by King.[12] Based on a kinetic theory of surface diffusion and a BET isotherm model for water in wool developed concurrently by Cassie,[13] King argued that D_0 should be inversely proportional to the square of the density of unoccupied adsorption sites on the keratin substrate. Thus, its value was predicted to rise abruptly as the primary water binding sites were filled. However, for keratin–water systems, the kinetic analysis is less than rigorous and the BET isotherm description has been found to be inadequate.[7] We discuss below three alternative theoretical approaches for estimating D_0 for water in a keratin matrix over the range of water concentrations characteristic of human SC *in vivo*.

Method 1. At sufficiently high levels of hydration, keratin microfibrils comprise impenetrable obstacles (radius r_2) impeding the Brownian diffusion of solute through a connected surrounding aqueous phase. The solute here comprises tracer water molecules, modeled as spheres of radius $r_1 = 1.1$ Å (vide infra). Considerable classical continuum theory exists on calculating effective (macroscopic average) diffusion coefficients D_0 for these types of structures, and it is worthwhile to see what orders of magnitude thereof are implied for the corneocyte interior by such theory.

Results on this problem for random fiber arrays were cast in a particularly useful and general form by Clague and Phillips.[14] Following a suggestion by Brady,[15] these authors noted that the outcome of a completely rigorous analysis[16,17] for certain periodic fiber configurations can be approximated reasonably by a factorization of the form

$$\frac{(D_0)_{\text{free}}}{D_{11}} = f_T(\varphi_2') \cdot \frac{\langle D_S \rangle}{D_{11}} \qquad (7)$$

(in somewhat different notation). The subscript "free" distinguishes freely diffusing solute and is discussed further and removed later. The first factor in eq. 7 is a "steric factor" representing the effective diffusivity of a hypothetical point-sized solute diffusing within the porous structure divided by its bulk diffusivity D_{11} in the continuous interstitial (here aqueous) fluid. This factor has been extensively studied since the pioneering analyses of macroscopic conduction properties of composite materials by Maxwell[18] and Rayleigh.[19] For a given type of arrangement of the fibers, f_T depends on the excluded volume fraction. The "prime" (′) affixed to the fiber volume fraction φ_2 is explained later. The second factor is a "short-time hydrodynamic coefficient" representing an average of the diffusivity of the actual solute, which varies with position because of varying proximity to the fibers, over the set of all accessible positions within the membrane. It is likewise made dimensionless with the bulk fluid diffusivity in the absence of the fibers, and depends on both the fiber volume fraction φ_2 and, more particularly, the ratio $\lambda = r_1/r_2$ of solute-to-fiber radii. This latter factor is analogous to the well-known hydrodynamic hindrance factor for diffusion in circular cylindrical pores, which is often modeled using the Renkin equation.[20] Although the Renkin equation might, in principle, be applied here with the introduction of an appropriate average pore size (which would be difficult to assess), it is inherently ill suited to modeling fibrous structures which have an unconsolidated solid phase and a non-convex pore space, at least at low volume fractions.

Concerning the first (steric) factor in eq. 7, Tomadakis and Sotirchos[21] presented an extensive computer simulation study of conduction in fibrous structures, including results for various types of random arrays of nonconducting, freely overlapping cylinders. (Their "formation factor" is the reciprocal of the effective conductivity/diffusivity.) The keratin microfibrils in a corneocyte are largely oriented in the plane of the skin, but form a random structure within the plane. The direction of motion for skin penetration is perpendicular to the fiber axes. Tomadakis and Sotirchos's[21] case of perpendicular transport through "random arrays of bi-directional nonconducting cylinders," represented in their Figure 4, corresponds exactly to this situation. As shown in that figure, their numerical results are well represented by a very simple formula due to Mottram and Taylor.[22] As considered here, the effective diffusivity is the ratio of macroscopic flux to gradient in superficial solute concentration in the membrane. "Superficial concentration" means the amount of solute, which is excluded from the fiber phase, per total volume of the system. To fit this convention, the effective conductivity results of Tomadakis and Sotirchos[21] and Mottram and Taylor[22] must be divided by a factor of the interstitial fluid volume fraction $\varphi_1' = 1 - \varphi_2'$. Thus, in the present context, Mottram and Taylor's formula[21,22] expresses itself as $f_T(\varphi_2') = \varphi_1' = 1 - \varphi_2'$.

Clague and Phillips[14] point out that the hypothetical point-sized solute involved represents the center of the actual solute, which cannot approach the cylindrical fiber axes more closely than a distance of $r_1 + r_2$. Thus, the effective excluded volume fraction φ_2' to be used in $f_T(\varphi_2')$ is actually larger than the fiber volume fraction φ_2. At small values of φ_2, for which the complicating geometrical phenomenon of cylinder overlaps is rare, φ_2' is just $\varphi_2\ (1+\lambda)^2$. As calculated later, water is characterized by a solute/fiber size ratio $\lambda = 0.03$, which renders the extra excluded volume, and the distinction between φ_2' and φ_2, quantitatively unimportant.

Concerning the second (hydrodynamic) factor in eq. 7, Clague and Phillips'[14] Figures 7 and 8 present, *inter alia*, detailed calculations of $\langle D_S \rangle / D_{11}$ over the range $0.5 \le \lambda \le 10$ for dilute fiber arrays ($\varphi_2 \le 0.06$), showing significant hindrance ($\langle D_S \rangle / D_{11} \approx 0.3$) for the largest values of λ and φ_2 considered. (The preceding statements are

translated into the present notation; Clague and Phillips's λ is actually the reciprocal of our λ). The hydrodynamic factor $\langle D_S \rangle / D_{11}$ should theoretically approach unity in the limit of a point-sized solute ($\lambda \to 0$). Hydrodynamic hindrance is therefore likely to be relatively unimportant for a solute molecule as small as water, again because $\lambda = 0.03$. Thus, we believe it is reasonable to take $\langle D_S \rangle / D_{11} \approx 1$ for water. This statement is supported by the trend of Clague and Phillips's results,[14] and also a detailed quantitative assessment[23] of hydrodynamic hindrance effects assembling the best available theory of Phillips et al.[14,16,17] and others, according to which $\langle D_S \rangle / D_{11} > 0.93$ for $\varphi_2 < 0.64$ (with $\lambda = 0.03$).

Based on the preceding discussion, an upper limit on water mobility within well-hydrated corneocyte interiors is given by the equation

$$\frac{(D_0)_{\text{free}}}{D_{11}} \approx \varphi_1 = 1 - \varphi_2 \qquad (8)$$

Extra solute size-related exclusion and hydrodynamic hindrance are likely to have relatively small effects for water. The salient conclusion to be drawn from this equation is that the presence of keratin fibers as geometrical obstacles does not dramatically reduce the intrinsic mobility of water from its bulk value.

Method 2. Equation 8 applies to water molecules that are entirely free to move. As discussed in the accompanying paper,[7] however, binding interactions of various strengths exist between water and the keratin fibers. Such interactions tend to slow diffusion because solute (tracer water) is more or less immobile in various bound states. General theory exists for the incorporation of adsorption phenomena and energetic interactions into effective diffusivity calculations for periodic microscopic models of porous media.[24,25] Rigorous applications thereof have been restricted to a few idealized structures,[25] and physically realistic calculations would necessarily be highly system specific. Development of a detailed microscopic model for the water–keratin system (e.g., on a par with the analyses of Phillips and co-workers,[14,16,17]) is a subject for future investigation. Solute–fiber interactions and diffusion are intertwined on the microscopic scale, so one cannot generally expect to be able to modify results like eqs. 7 and 8 in a simple way (e.g., using a factorized formula similar to eq. 7) to account for bound states. Nevertheless, an approximate "add-on" of some existing results in the literature does provide valuable insights. In discussing these results, we adopt an approximate view of $(D_0)_{\text{free}}$ as a constant, to be modified, characterizing the intrinsic mobility properties of a hypothetical average membrane (water + keratin) continuum without any binding interactions.

The FHH equation (eq. 1) implies a picture in which the water–keratin interaction is described by a potential that varies with an inverse power of distance.[7] Thus, the real situation is not far removed from that of diffusion through a structure in which the solute has a position-dependent potential energy, ψ. Results on this type of problem exist for spatially periodic functions ψ in other contexts.[26,27] In the simplified case of a one-dimensional medium in which the solute has a periodic potential energy $\psi(x)$,[26,27]

$$D_0 = \frac{(D_0)_{\text{free}}}{\langle \exp(\psi(x)/kT) \rangle \langle \exp(-\psi(x)/kT) \rangle} \qquad (9)$$

The angle brackets ($\langle\rangle$) in eq. 9 denote the average over one period. The factor in the denominator is always ≥ 1,[26,27] and any spatial variations in energy (e.g., arising from an attractive interaction with a surface) reduce the effective diffusivity. For variations in ψ that are large compared with kT, eq. 9 exhibits Arrhenius-type asymptotic behavior,[27] and the reduction is dramatic. It is difficult to develop this qualitative statement further for the present problem.

An alternate, extreme "on-off" idealization of the actual situation might introduce a bound solute population with concentration C_{bound} coupled to a free (mobile) solute concentration C_{free} by a first-order reversible binding equilibrium, characterized by an equilibrium constant $K^{\text{binding}} = C_{\text{bound}}/C_{\text{free}}$. In this situation, it is well known[11,28,29] that the average diffusivity characterizing the motion of all (free + bound) solute is given by the formula

$$D_0 = \frac{(D_0)_{\text{free}}}{1 + K^{\text{binding}}} \qquad (10)$$

The manner in which an increase in K^{binding} slows diffusion is in accord with intuition.

At sufficiently high levels of hydration, the surfaces of the keratin microfibrils are likely to be separated from each other by a distance exceeding the range of any binding forces. It might therefore be reasonable to suppose that there exists an asymptotic volume of bound water (say V_{bound}) per unit of (isolated, noninteracting) fiber length in the limit $\varphi_2 \to 0$. This concept applies only to the

case of self- (tracer water) diffusion considered in this paper, for which the water concentration is constant ($\rho_1 \approx 1$ g/cm^3) far from any fiber. It also leaves unaddressed the thermodynamic processes by which a given water activity produces a certain degree of hydration and therefore water volume fraction φ_1 (cf. eq. 1). Introducing free and bound water volume fractions [$(\varphi_1)_{free}$ and $(\varphi_1)_{bound}$, respectively], it follows that $(\varphi_1)_{bound} = L_{fiber}V_{bound}$, where $L_{fiber} = \varphi_2/\pi r_2^2 = (1 - \varphi_1)/\pi r_2^2$ is the mean length of fibers contained within a unit of total (water + fiber) membrane volume. Since $(\varphi_1)_{free}$ and $(\varphi_1)_{bound}$ must add up to φ_1, it follows that

$$(\varphi_1)_{free} = \varphi_1 - (\varphi_1)_{bound} = \varphi_1 - \kappa(1 - \varphi_1) \quad (11)$$

and

$$K^{binding} = \frac{(\varphi_1)_{bound}}{(\varphi_1)_{free}} = \frac{\kappa(1 - \varphi_1)}{\varphi_1 - \kappa(1 - \varphi_1)} \quad (12)$$

in which $\kappa = V_{bound}/\pi r_2^2$ is an adjustable binding parameter. Unlike eq. 8, which is quantitatively accurate even at relatively large values of the fiber volume fraction,[21] eqs. 11 and 12 are asymptotic results that are restricted to the limit where the water–keratin interaction occurs in effective isolation for each fiber.

Equation 12 can be substituted into eq. 10 yield a formula for D_0 accounting approximately for not only the geometrical (obstacle) but also the binding properties of the fibers. In calculating $(D_0)_{free}$ in eq. 10 using eq. 8, φ_1 must be replaced by $(\varphi_1)_{free}$ (eq. 11) because bound water contributes to the effective size of the obstacles. The final result of the preceding, extremely simple binding model is the following equation:

$$\frac{D_0}{D_{11}} = \frac{[\varphi_1 - \kappa(1 - \varphi_1)]^2}{\varphi_1} \quad (13)$$

which is reasonable for sufficiently small fiber volume fractions and contains one adjustable binding (or affinity) parameter κ.

The presence of natural moisturizing factor and other dissolved chemical species could conceivably make the viscosity of the intracorneocyte aqueous phase somewhat larger than that of pure bulk water, correspondingly decreasing the value of D_{11}. The osmotic contribution of natural moisturizing factor is certainly the second factor (in addition to the chemical affinity of keratin for water) driving the enormous water-holding capacity of SC (see Ref. 7 and references therein). This phenomenon does not materially change our analysis or conclusions.

Method 3. An alternative approach is provided by a theory developed by Yasuda and co-workers[30,31] to explain water mobility in swollen hydrogels. Based on a free volume analysis, these workers proposed an equation for water diffusivity D_0 as a function of volume fractions of water (φ_1) and polymer (φ_2) of the form

$$\ln\left(\frac{D_0}{D_{11}}\right) = -\beta\frac{Y(1 - \alpha)}{1 + \alpha Y} \quad (14)$$

where

$$Y = \frac{\varphi_2}{\varphi_1} = \frac{1 - \varphi_1}{\varphi_1} \quad (15)$$

In eq. 14, α is the ratio of free volume in the dry membrane to that in bulk water, β is the ratio of the critical diffusion volume of the permeant to the free volume in bulk water, and D_{11} is the self-diffusion coefficient of water. We will apply these analyses to estimate D_0 in the corneocyte phase of the SC.

Calculation of Water Transport

Consider a keratin membrane of thickness h (e.g., a nail or an isolated corneocyte) in contact with two external media having water activities a_1 and a_2. Water transport across the membrane is calculated from eq. 2 with $D(C)$ calculated as in eq. 4. The concentration gradient $\partial C/\partial z$ is calculated from the solution of the diffusion equation

$$\frac{\partial C}{\partial t} = \frac{\partial}{\partial z}\left(D(C)\frac{\partial C}{\partial z}\right) \quad (16)$$

within the membrane, subject to the boundary conditions $a_w(0) = a_1$, and $a_w\ (h) = a_2$. These boundary values are translated into in-membrane water volume fractions $\varphi_1(0)$ and $\varphi_1(h)$ [numerically equal to concentrations $C(0)$ and $C(h)$] using the appropriate sorption isotherm. For SC keratin, this relationship is given by eqs. 1 and 5. The problem, although not simple, is well-defined and can be solved by numerical methods.[32] Similarly, water transport across a multilaminate lipid/corneocyte barrier such as SC could be calculated by this approach, given a suitable model for water permeability of the lipid phase.

A simpler case to consider is that of a membrane surrounded by two identical solutions. In this case, water activity is constant throughout the system [a fact which effectively eliminates the second

(thermodynamic) factor in eq. 4], and there is no net transport at steady state. However, the permeability of the membrane to water may be measured by a tracer experiment. The steady-state flux of the tracer is described by

$$J_{ss} = -D_0 K \frac{\Delta C_{ext}}{\Delta z} = -P \frac{\Delta C_{ext}}{\Delta z} \qquad (17)$$

where $\Delta C_{ext}/\Delta z$ is the difference in bulk-equivalent concentrations of the tracer external to a layer of the membrane of thickness Δz and K is its membrane–solution partition coefficient. A discontinuous change in activity coefficient at the membrane–solution interface and/or a possible porosity factor (cf. eq. 18) is included in K, and $D = D_0$ within the membrane.[11] The quantity $P = D_0 K$ is known as the diffusive permeability.[30] For aqueous solutions in contact with a water-swollen membrane, the partition coefficient for water is approximately φ_1.[30] Thus

$$P = D_0 \varphi_1 \qquad (18)$$

We will use the aforementioned definition for P and reserve the alternate term of permeability coefficient, k_p, for the related quantity

$$k_p = -\frac{J_{ss}}{\Delta C_{ext}} = \frac{P}{\Delta z} \qquad (19)$$

For a homogeneous membrane of thickness h, $k_p = P/h$. The diffusive resistance of the membrane, $R_m = 1/k_p$, is simply the inverse of the permeability coefficient. For a multilaminate membrane, the total diffusive resistance may be obtained by summing the resistances of the separate layers, each having its own thickness Δz.[33]

EXPERIMENTAL

Diffusivity Estimation

Method 1

An upper limit on water diffusivity in the corneocyte phase of the SC was estimated from eq. 8. The self-diffusion coefficient of water D_{11} was taken to be 2.7×10^{-5} cm^{2}s^{-1}. This value was calculated by adjusting the 25°C value cited by Yasuda et al.[30] (i.e., 2.44×10^{-5} cm^{2}s^{-1}) to 30°C by multiplying by the inverse viscosity ratio at these temperatures. In developing this approximation, the radius of a water molecule r_1 was taken to be 1.1 Å (the Stokes–Einstein radius) and that of a keratin microfibril r_2 to be 35 Å,[34] giving $\lambda = r_1/r_2 = 0.03$. The fact that $\lambda \ll 1$ supports our tentative neglect of hydrodynamic hindrance effects on intrinsic free water mobility in eq. 8.

Method 2

The value of the binding parameter κ in the modified composite continuum theory (eq. 13) was adjusted to provide the best fit to two pulsed-field-gradient spin-echo nuclear magnetic resonance (NMR) measurements of mobile proton diffusivity in guinea pig footpad SC.[35] These data are shown in Table 1 and interpreted later. Like Method 1, Method 2 is only appropriate in high hydration regimes where the keratin microfibrils are surrounded by a continuous water phase; thus, no attempt was made to include the wool and horn data in the estimation of κ.

Method 3

Water diffusivity data in wool[36] and horn[12] keratin derived at 35 and 25°C, respectively, were adjusted to 30°C using the Arrhenius equation and activation energies, E_a, reported in the original references. The original and adjusted diffusivities are shown in Table 1. Water content in these reports is expressed as regain, which is the percentage of water "regained" by the substrate when a dried sample is equilibrated in air at a specified relative humidity. In both cases, the value of E_a was taken to be 11 kcal mol^{-1} for zero regain, 5 kcal mol^{-1} for regains >10%, and a linear interpolation of these values for regains in the range 0–10%.[12,36] This procedure yielded temperature-adjusted diffusivities 12–25% lower than the reported values for wool and 15% higher than those reported for horn. Regains ($\%R$) were converted to volume fraction water φ_1 and the related hydration parameter Y using eqs. 5 and 15, with $\nu = \%R/100$ and $\rho_{mem} = 1.3$ g/cm^3.[1] The free volume expression for diffusivity in swollen polymer systems, eq. 14, was fit to these data by nonlinear regression, assuming the aforementioned value of D_{11} for the self-diffusion coefficient of water.

RESULTS

Water diffusivity data in wool and horn keratin, adjusted to 30°C, are shown in Figures 1 and 2. Also plotted are the two spin-echo NMR measurements for mobile protons in guinea pig footpad

Table 1. Water and Mobile Proton Diffusivities in Horn, Wool, and Guinea Pig Footpad Stratum Corneum

% Regain[a]	φ_1[b]	$D(T) \times 10^9$, $cm^2 s^{-1}$	E_a, kcal mol^{-1}	$D(30°C) \times 10^9$, $cm^2 s^{-1}$
Horn (25°C)[c]				
7.3	0.087	1.3	6.6	1.6
10.9	0.124	14	5	16
14.3	0.157	52	5	60
15.6	0.169	91	5	104
Wool (35°C)[d]				
1	0.013	0.19	10.4	0.14
2	0.025	0.27	9.8	0.21
3	0.038	0.35	9.2	0.27
4	0.049	0.57	8.6	0.45
5	0.061	0.93	8.0	0.75
6	0.072	1.6	7.4	1.3
8	0.094	3.4	6.2	2.8
10	0.115	4.4	5	3.9
12	0.135	5.1	5	4.4
14	0.154	5.4	5	4.8
16	0.172	5.8	5	5.1
18	0.190	6.0	5	5.2
Guinea pig SC (unspecified temp)[e]				
33	0.300	1200	5	1200
59	0.434	2800	5	2800

[a] (g H_2O/g dry tissue) × 100%. For definition, see text.
[b] Water volume fraction.
[c] Reference 12.
[d] Reference 36.
[e] Reference 35.

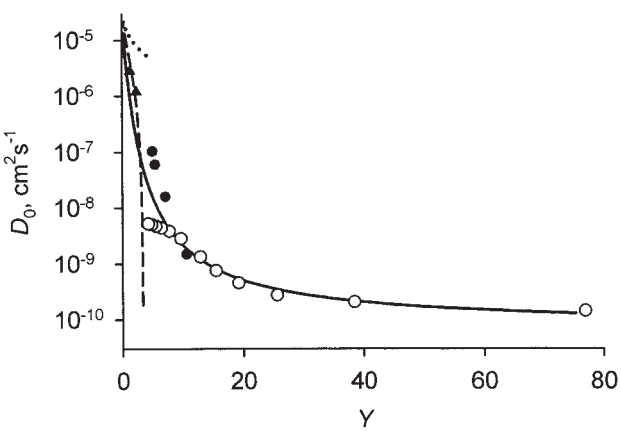

Figure 1. Unadjusted water and mobile proton diffusivities in horn, wool, and guinea pig footpad SC as a function of the keratin/water volume fraction ratio $Y = \varphi_2/\varphi_1 = (1 - \varphi_1)/\varphi_1$.[30] Key: (●) horn–water system[12]; (○) wool–water system[36] (▲) mobile protons in guinea pig SC.[35] The solid curve is a fit of the free volume relationship (eq. 14) to these data, yielding $\alpha = 0.278$, $\beta = 4.94$, and $r^2 = 0.77$. The dotted curve represents the classical composite continuum relationship (eq. 8) and the dashed curve is the modified composite continuum relationship including binding (eq. 13), plotted using $\kappa = 0.30$.

SC.[35] The theoretical curves in these figures correspond to the aforementioned diffusivity estimation methods. The classical composite continuum model (Method 1) represents an upper limit containing no adjustable parameters, whereas the

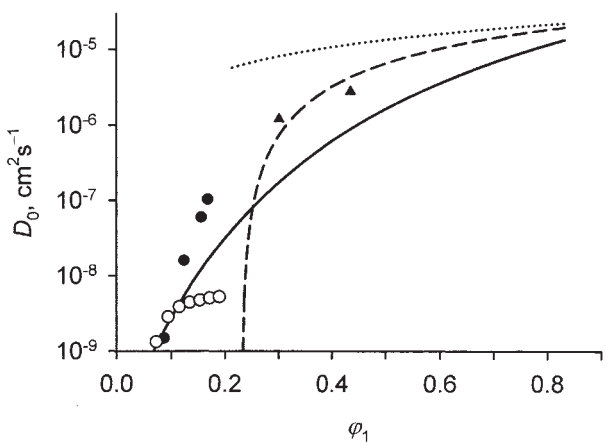

Figure 2. Unadjusted water/mobile proton diffusivities in horn, wool, and guinea pig footpad SC plotted as a function of the water volume fraction φ_1. Data and theoretical curves are identified as in Figure 1.

free volume model (Method 3) represents a fit to the low water content (i.e., wool and horn) data using two adjustable parameters, α and β. The modified composite continuum model (Method 2) represents a fit to the high water content (guinea pig SC) data containing a single adjustable parameter, κ. Methods 1 and 2 are only expected to be applicable in the hydration regime in which the fibers are embedded in a continuous liquid matrix; for example, the regime corresponding to water volume fractions $\varphi_1 \geq 0.21$ or $Y \leq 4$ if one assumes a square array of cylindrical fibers. At low water contents, Method 1 leads to diffusivities much larger than those measured in other keratinized tissues or calculated from the free volume approach. Method 2 is similar to Method 1 for small values of κ, but falls abruptly before rising steeply at low water content for larger values of κ. Such behavior is beyond the range of validity of the asymptotic formulas (eqs. 11 and 12). Hence, neither Method 1 nor Method 2 should be considered in the low water content regime.

The curve corresponding to the free volume model was calculated using the parameters $\alpha = 0.278$ and $\beta = 4.94$. The value of β is close to the value of 4.5 estimated for water in synthetic polymer hydrogels.[30,31] This is satisfying because β is a property of the permeant and solvent only, which are water in both examples. The value of α is less than the value 0.5 estimated for the hydrogel systems, suggesting that the free volume of the dry keratin is less than that of the synthetic polymers in Yasuda's study[30]. A more chemical explanation of this result is that water interacts with keratin much more strongly than with cellulose or acrylic acid esters; thus, its mobility in the dry polymer is much less. A comparison of unadjusted water diffusivity values D_0 at low hydration levels (large Y) shows that D_0 is >1000-fold lower in keratin than in the synthetic polymers.

The water content of SC keratin *in vivo* is typically much greater than that of the wool and horn keratins shown in Figures 1 and 2. Hence, use of the free volume model to describe water diffusivity in SC keratin based on these data involves a long interpolation. It is interpolation rather than extrapolation because the curve must end at D_{11} for a 100% water system. Additional justification for such an interpolation is provided by the mobile proton diffusivity values in guinea pig footpad (Figures 1 and 2), which were obtained on hydrated plantar SC. Packer and Sellwood concluded that these values represent the diffusivity of water and other water-soluble compounds (e.g., natural moisturizing factor components) diffusing within the corneocyte.[35] The investigators find no reason to question this conclusion. Consequently it is reasonable to assume that unadjusted water diffusivity $D_0 = D_0(\varphi_1)$ in partially hydrated SC keratin can be approximated by the aforementioned methods. One can then estimate the thermodynamically adjusted diffusivity D and permeability P as a function of SC water activity a_w by combining $D_0(\varphi_1)$ with eqs. 1–6 and 18, respectively. For example, eqs. 1 and 5 can be used to estimate φ_1 at any value of a_w; then, eqs. 4 and 6 can be used to calculate D. The results of such an analysis, based on the free volume diffusivity model, are shown in Figures 3 and 4. This analysis suggests that the value of D in skin keratin increases almost 300-fold between 10 wt % hydration ($a_w = 0.67$) and 79 wt % hydration ($a_w = 1$), and the value of P increases by more than 10^4. The question of whether or not a change of this magnitude could lead to a change in the predominate penetration pathway is discussed later.

DISCUSSION

The diffusivity and permeability values just presented for water in corneocytes are not directly confirmable from macroscopic SC water transport

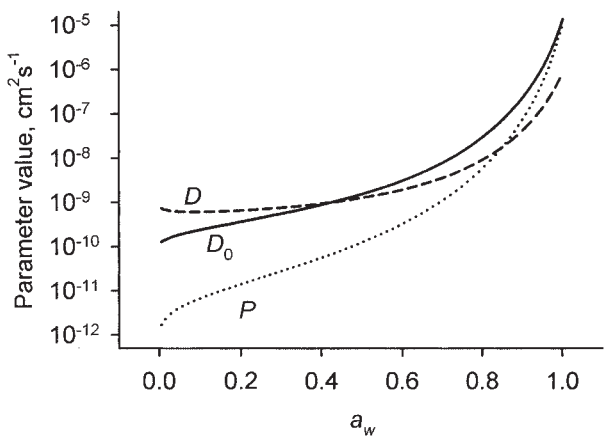

Figure 3. Unadjusted and thermodynamically adjusted water diffusivities D_0 and D and permeability P in SC keratin as a function of water activity a_w, based on the free volume model for D_0, eq. 14. The curve for D is calculated from eqs. 4 and 6 and that for P from eq. 18, using the equilibrium water sorption isotherm given in eq. 1. For high values of water activity ($a_w > 0.8$), somewhat higher values as estimated by the modified composite continuum theory (eq. 13) may be more accurate than those estimated from eq. 14 (cf. Figure 2).

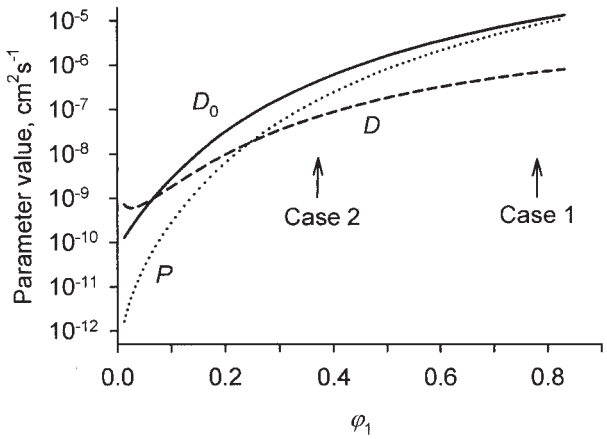

Figure 4. Unadjusted and thermodynamically adjusted water diffusivities D_0 and D and permeability P in SC keratin as a function of water volume fraction φ_1, based on the free volume model for D_0, eq. 14. The curves are those from Figure 3. Cases 1 and 2 are the hydration states considered in the Discussion.

measurements because of the profound influence of the intercellular lipids. Nevertheless, they are consistent with spectroscopic evidence. The latter has led investigators to describe two forms of bound water in SC, primary and secondary.[37,38] Both forms may be distinguished by infrared (IR) analysis, whereas NMR distinguishes only one.[37] Primary bound water is considered in the older literature to be associated with the polar functional groups of keratin and other skin proteins;[37,38] more recent accounts include the water of hydration of intercellular lipid headgroups.[6,39] The primary form is essential for SC pliability.[40] According to Spencer,[38] the first 0.10–0.15 g H_2O/g dry tissue at 60% relative humidity constitutes primary bound water, although more primary binding sites are evidently exposed at high levels of hydration.[37] Thus, the BET monolayer value determined in the accompanying paper,[7] $\nu_m = 0.0386$ g H_2O/g dry tissue, reflects only a fraction of the primary bound water in SC.

Hansen and Yellin[37] proposed that water molecules bound to primary and secondary sites in SC exchange rapidly on an NMR time scale (10^{-8} s), but slowly on an IR time scale (10^{-14} s), making them indistinguishable by the former technique, but distinguishable by the latter. The spin–spin relaxation curves for water in hydrated SC containing 0.50–1.5 g H_2O/g dry tissue could be fit by assuming two species of water, one (the less mobile fraction, ~0.30 g H_2O/g dry tissue) with a relaxation time τ_2 of 17 ms, the other with $\tau_2 = 106$ ms. These values are intermediate between the values for ice ($\tau_2 = 30$ μs) and for bulk liquid water at 30°C ($\tau_2 \approx 3$ s). Thus, even the "free" water in SC at these levels of hydration has restricted mobility compared with the bulk liquid. We note that the value $\kappa = 0.30$ determined by diffusivity estimation Method 2 (cf. Figs. 1 and 2) is in substantial agreement with Hansen and Yellin's result of 0.30 g H_2O/g dry tissue for the less mobile species. This agreement may be seen by noting that κ is the ratio of the volume of bound water to volume of fiber (cf. eqs. 11 and 12), corresponding to within a factor of fiber density (plus a small correction for SC lipid mass) to Hansen and Yellin's mass ratio. Expressing the result in terms of concentric cylinders, this value of κ corresponds to a layer of bound water ~5 Å thick surrounding each keratin microfibril of radius $r_2 = 35$ Å. Using an effective radius $r_1 = 1.1$ Å for the water molecules translates into a bound water layer averaging 2.3 molecules in thickness. However, based on the conformity of SC water sorption data to the FHH isotherm with a low value of n and the associated implication of long-range intermolecular forces,[7] it seems reasonable to consider a continuous spectrum of water mobilities and relaxation times in SC rather than two discrete species. Thus, the on–off binding model represented by eqs. 10–13 and already discussed very likely oversimplifies the true situation.

Using a combination of relaxation measurements and pulsed field-gradient spin–echo NMR techniques, Packer and Sellwood[35,41] studied the motion of mobile protons in guinea pig footpad SC. Water protons were distinguished from nonaqueous protons by hydrating dry tissue samples with H_2O and D_2O. Both types of protons made substantial contributions to the observed signal. The nonaqueous mobile protons were postulated to be associated with natural moisturizing factor components (amino acids, urea, lactate, pyrrolidone-2-carboxylate) and with lipids. In hindsight, it seems that the SC lipids may not have contributed because they are highly organized into lamellar bilayers, [34] however, this was not known at the time. It is clear (also in hindsight) that the substantial signal associated with water protons must arise primarily from water within the corneocytes because the amount is much greater than that associated with lipid headgroups in hydrated SC.[39] This fact appears to have been overlooked by others interpreting these data,[6] who have construed the reported diffusivity values to apply to extracellular water. In our estimation, this

explanation cannot be the case. The conclusion attributed to Packer and Sellwood that the corneocyte is impermeable[6] is that of the analysts rather than the original investigators.

Among the several findings from the guinea pig footpad NMR studies[35,41] were the following: (1) Spin–lattice relaxation for mobile protons was not a simple exponential process for any degree of hydration, suggesting multicomponent behavior. This suggestion is consistent with the findings of Hansen and Yellin.[37] (2) The aqueous and nonaqueous mobile protons diffused at comparable rates of $\sim 10^{-6}$ $cm^2 s^{-1}$. (3) The distances over which these protons diffused in intact SC were restricted to 3–4 μm in the transverse direction (perpendicular to the plane of the SC) and 10–20 μm in the longitudinal direction. (4) Extraction of the SC with 2:1 chloroform/methanol substantially reduced the effectiveness of the restricting barriers, especially in the transverse direction. Packer and Sellwood[35] attributed the restrictions to the "cell walls" of the corneocytes, but the structure of these barriers had not yet been worked out. In retrospect, we surmise that they must have been either the cornified cell envelopes, the intercellular lipids, or a combination of both.[34] The fact that barrier efficiency was greatly reduced by chloroform/methanol extraction, which extracts all noncovalently bound lipid but leaves the cornified cell envelope intact,[34] strongly suggests the restriction was associated with intercellular lipid lamellae. The remaining cornified cell envelopes were evidently not a substantial water barrier. This interpretation will become important in the analysis of SC water barrier location described later.

Implications for SC Permeability

The water diffusivity estimates in Figures 1–4 apply to the interior regions of the corneocyte, which is mostly keratin. The corneocyte is bounded by a cornified cell envelope that is ~15 nm thick and comprised of loricrin, involucrin, and other structural proteins, to which a layer of lipid is covalently bound.[34] The intercellular regions of the SC are filled with lamellar lipids.[34] Furthermore, the water activity gradient across the SC *in vivo* imposes a depth-dependent water content within the corneocytes. The macroscopic permeability of the tissue is determined by the composite properties of these regions, hence would not be expected to change in proportion to the values in Figures 3 or 4. However, it is instructive to consider the permeability of the composite membrane in two different limits. To do this we let R_c, R_{ce}, and R_l represent the diffusive resistance of the corneocyte interiors, the cornified cell envelopes, and the intercellular lipids, respectively. If $R_c + R_{ce} \ll R_l$, the lipid resistance dominates and the SC may be treated as a multilaminate membrane in which $R_{tot} = R_c + R_{ce} + R_l$. If $R_c + R_{ce} \approx R_l$, both phases are important, but diffusion is primarily transcellular because the area fraction of corneocytes in an SC layer is so much greater than the area fraction of lipids. It is not until $R_c + R_{ce} \gg R_l$ that the intercellular lipid pathway becomes important. From Heisig's two-dimensional analysis of a fully-staggered (i.e., maximally unaligned) corneocyte matrix, one may infer that the ratio P_l/P_c must exceed 1000 for the latter to occur.[42] Because the aggregate corneocyte thickness h_c is 10- to 50-fold greater than the lipid thickness h_l, the ratio $(R_c + R_{ce})/R_l$ must exceed 10^4 for intercellular permeation to play a significant role in a two-dimensional lattice. Although a different value may apply with partially aligned corneocytes[43] in three dimensions, it is not likely to vary from Heisig's result by orders of magnitude. This position is supported by ongoing analysis in our laboratories.[23]

The free volume diffusivity estimate (Method 3) was chosen for the water transport analyses shown next. Of the alternatives considered, this method led to the lowest estimates for water diffusivity in hydrated keratin and, correspondingly, the greatest contribution of the corneocyte phase to the total diffusive resistance of the SC.

Case 1. Fully Hydrated SC

The water content for SC equilibrated with normal saline, $a_w = 0.996$, is estimated from eq. 1 be $v = 2.75$ g H_2O/g dry tissue, corresponding to a volume fraction water $\varphi_1 = 0.78$. Unadjusted water diffusivity in the intracellular keratin phase at this water content and 30°C is estimated from Figure 4 be $D_0 = 1.07 \times 10^{-5}$ $cm^2 s^{-1}$, which is only 2.5-fold less than the value in pure water. The partition coefficient for water between normal saline and keratin is simply equal to φ_1; hence, the diffusive permeability of the corneocyte interiors $P_c = D_0 K$ is equal to 0.83×10^{-5} $cm^2 s^{-1}$ (cf. eq. 18). Assuming 15 corneocyte layers in the SC and a dry thickness of 13 μm,[44] one can estimate the swollen SC thickness to be ~57 μm.[45] Of this value, the corneocytes would comprise 56 μm and the lipids 1 μm because the lipid regions do

not swell appreciably.[39] The diffusive resistance of the corneocyte interiors is thus $R_c = h_c/P_c = (56 \times 10^{-4}\ \text{cm})/(0.83 \times 10^{-5}\ \text{cm}^2\text{s}^{-1}) = 670$ s/cm or 0.19 h/cm.

Taking the water permeability coefficient of hydrated SC to be $k_p = 1 \times 10^{-3}$ cm/h,[33] the diffusive resistance of the composite membrane is $R_{tot} = 1000$ h/cm. Because $R_c \ll R_{tot}$, the interior keratin phase contributes very little to the composite resistance. Unless the cornified cell envelopes contribute significantly to the barrier, the cells are essentially transparent.

How impermeable would the cornified cell envelopes have to be to significantly impact water transport? Their total thickness h_{ce} is $\sim 30 \times 15\ \text{nm} = 450$ nm. A diffusive permeability P_{ce} of $1 \times 10^{-10}\ \text{cm}^2\text{s}^{-1}$—five orders of magnitude less than P_c—leads to $R_{ce} = h_{ce}/P_{ce} = (4.5 \times 10^{-5}\ \text{cm})/(1 \times 10^{-10}\ \text{cm}^2\text{s}^{-1}) = 4.5 \times 10^5$ s/cm or 125 h/cm. This value is still only one-eighth of the total SC resistance, R_{tot}. To exceed Heisig's criterion for significant intercellular transport, $(R_c + R_{ce})/R_l > 10^4$ (vide supra), P_{ce} would have to be $< 1 \times 10^{-15}\ \text{cm}^2\text{s}^{-1}$. Such a value is well below the range expected for hydrophilic polymers.[10] Furthermore, the proton NMR studies already described[35] suggested that intercellular lipids rather than cornified cell envelopes provide restrictions to water transport in guinea pig footpad SC. These arguments suggest that transcellular permeation dominates water transport in hydrated SC.

Case 2. Partially Hydrated SC

Consider a sample of SC with an average water content of 30 wt %, approximately equal to the *in vivo* level. Neglecting the gradient present *in vivo*, the equilibrium parameters associated with this hydration level (cf. Ref. 7 and Figure 4) are $a_w = 0.92$, $\nu = 0.45$, $\varphi_1 = 0.37$, $D_0 = 4.3 \times 10^{-7}\ \text{cm}^2\text{s}^{-1}$ and $P_c = 1.6 \times 10^{-7}\ \text{cm}^2\text{s}^{-1}$, based on the FHH isotherm and the free volume diffusivity approach. From the water content one estimates a total SC thickness of 20 μm,[45] resulting in $h_c = 19$ μm. This value leads to a calculated value of $R_c = 3.3$ h/cm, which is still much less than the resistivity of hydrated tissue, $R_{tot} = 1000$ h/cm, calculated in the previous example. However, partially hydrated SC is less permeable (thus more resistive) to water than fully hydrated SC by a factor of at least 3–4.[1] Evidently, although the resistivity of the corneocyte phase is greater than in fully hydrated SC, the magnitude of the change is not sufficient to explain an increase in resistivity of the tissue as a whole. This result implies that an increase in the resistivity of the lipid phase is also involved, as has been proposed by others.[6,39] By an argument similar to that in Case 1, most water transport in Case 2 is still likely to be transcellular.

The conclusion regarding the water transport pathway in SC is strikingly different from that of Johnson et al.,[44] who argued that lateral diffusion through the SC lipids (a form of intercellular transport) was the primary transport mechanism for most compounds through SC. These findings are not entirely incompatible, because the database analyzed by Johnson et al. (an extension of the Flynn database[46]) consisted largely of lipophilic compounds for which the intercellular transport mechanism is plausible. However, the findings do conflict for water. Johnson et al.[44] held that water transport could be described by the lateral diffusion model, with a diffusivity of $\sim 1.9 \times 10^{-5}\ \text{cm}^2\text{s}^{-1}$. This idea presented some problems because the diffusivity was close to the self-diffusivity value—surprising for water associated with polar lipid headgroups—and the calculated lag time for achievement of steady state diffusion, t_{lag}, was only 0.3 s. Experimental lag times for water diffusion in hydrated SC taken from large body surfaces are ~10–20 min.[47] It is shown in the Appendix that the calculated value of t_{lag} for the transcellular permeation model already presented for hydrated SC is ~40 min. Thus, the transcellular transport model is more consistent with experimental lag times and with the water-in-keratin diffusivity data presented earlier.

A recent analysis of the lag times associated with lipid phase transport in two-dimensional brick-and-mortar SC models[48] suggests that the calculation of Johnson et al.[44] substantially underestimates the lag time associated with these structures. Using the method suggested by Frasch and Barbaro,[48] a hydrated SC thickness of 57 μm and the water diffusivity value from Johnson et al.[44], the authors calculate a lag time for water transport across the SC of ~2–3 s according to the lateral diffusion model. This result reduces, but does not eliminate, the discrepancy with the experimental values. According to this calculation, the value of the lag time is highly sensitive to the dimensions of the corneocyte and lipid regions but relatively insensitive to corneocyte arrangement within the two-dimensional lattice.

We note that a lateral diffusion model for water in SC had been proposed earlier by Potts and

Francouer.[6] This calculation has a flaw in the manner in which the lag time is related to the tortuous diffusion pathway through the SC, as may be seen by comparison with the works of Johnson et al.[44] or Frasch and Barbaro.[48] Hence, the calculation should not be used for quantitative work. It is interesting that the experimentally determined lag time of 8400 s (140 min) reported by these investigators for vapor phase water transport through porcine SC can be reconciled with human SC data and the transcellular diffusion model by assuming the porcine SC was 2–3 times thicker than human SC, as is frequently reported. This comparison is only qualitative because the porcine SC was only partially hydrated in this study. Alternatively, it is also possible that the observed lag time was influenced by the lag time for transfer of ^3H_2O between the solution and the vapor phase, which was not reported in the article.

In light of the analysis just presented, it is intriguing to consider the implications of the diffusivity models for permeants other than water. The composite continuum approach, eqs. 8–13, is readily adapted to other permeants by changing the solute radius, r_1, and judiciously applying existing results on steric[21] and hydrodynamic hindrance[14,16,17] factors. For solutes that interact significantly with the keratin microfibrils, calculating more than an upper limit would require at least the introduction of an empirical binding factor (cf. eq. 10) in the absence of a detailed microscopic model. The free volume model, eq. 14, contains two adjustable parameters, α and β. The former is a property only of the membrane and the hydrating solvent; the latter is proportional to the critical diffusion volume of the permeant. If the theory applies for water, one might anticipate it applying it to other permeants dissolved in water. The value of β, in this case, should vary in proportion to the size of the permeant and the diffusivity relative to that in water should scale as $e^{-\beta}$ (cf. eq. 14). This form is similar to the free volume model employed by Potts and Guy[46] and others to describe diffusivity in SC lipids. This conjecture seems worth testing for those permeants whose entrance into corneocytes can be established.

CONCLUSIONS

Water diffusivity and mobility in the keratin phase of the SC, as estimated from two independent approaches (Methods 2 and 3), increase sharply with increasing SC water content. For fully hydrated SC they are projected to be within a factor of 2–3 of the values expected for self-diffusion in pure water. The results suggest that the corneocyte phase is not a significant component of the SC water permeability barrier. Thus, water diffusion through the SC is likely to occur primarily by transcellular pathways in which the intercellular lipids provide most of the diffusive resistance.

ACKNOWLEDGMENTS

We thank Robert Laughlin for a helpful discussion and the reviewers for valuable suggestions for improving the clarity of the manuscript. Financial support from the Procter & Gamble Company's International Program for Animal Alternatives, NSF GOALI grant BES-9818160, NIOSH grant R01 OH007529, and the University of Cincinnati Research Council are gratefully acknowledged.

APPENDIX

Lag Time Estimate for Transcellular Water Transport through SC

The following estimate is based on known lag times for multilaminate membranes,[49] using SC dimensions and properties discussed in this report and elsewhere.[44] This picture, like a purely lateral diffusion model, is an oversimplification for the SC membrane, which has both lipid-continuous and water-continuous diffusion pathways in addition to the transcellular route.[44] The composite structure is likely to exhibit shorter lag times than the pure lipid/protein multilaminate structure assumed here[47]; nevertheless, this is a reasonable starting point if transcellular diffusion is indeed the dominant pathway. We begin with an expression tabulated by Barrer[49] for the lag time of a planar membrane consisting of a large number n of identical pairs of layers, designated l for lipid and c for corneocyte. It is acceptable to use the limit as $n \to \infty$ because the value of n is $\sim$15 for SC, and the corrections for finite n vary as $1/n^2$. A slight rearrangement of Barrer's eq. 29 yields

$$t_{\text{lag}}^{\infty} = \frac{1}{6}\left(\frac{h_l}{D_l} + K_{l/c}\frac{h_c}{D_c}\right)\left(h_l\frac{h_c}{K_{l/c}}\right) \qquad \text{(A-1)}$$

The total thickness of the membrane is $h = h_l + h_c$. The values are the unadjusted diffusivities (D_0) in each phase and $K_{l/c}$ is the lipid–corneocyte partition coefficient. Using the properties estimated for hydrated SC under Case 1 of the Discussion, one finds

$$h_l = 15 \times 0.075\,\mu m^{44} = 1.12\,\mu m = 1.12 \times 10^{-4}\,cm \quad \text{(A-2)}$$

$$h_c = h - h_l = 57\,\mu m - 1.1\,\mu m = 56\,\mu m = 56 \times 10^{-4}\,cm \quad \text{(A-3)}$$

$$D_c = 1.07 \times 10^{-5}\,cm^2\,s^{-1} \quad \text{(A-4)}$$

$$K_{l/c} = K_l/K_c = K_l/\varphi_1 = K_l/0.78 \quad \text{(A-5)}$$

$$P_l = D_l K_l \approx h_l \times k_p = (1.12 \times 10^{-4}cm) \times (1 \times 10^{-3}cm/h) \times 1\,h/3600\,s = 3.11 \times 10^{-11}cm^2s^{-1} \quad \text{(A-6)}$$

In eq. A-6 we have used the argument developed in Case 1 that the diffusive resistance of the lipids $R_l = h_l/P_l$ is approximately equal to the total SC resistance $R_{tot} = 1/k_p$. To complete the calculation one must independently estimate either D_l or K_l. This procedure is somewhat arbitrary because, unlike their product P_l, these values tend to be model dependent. Choosing the model for K_l from Johnson et al.,[44] which is similar to that of Potts and Guy,[46] the partition coefficient of water between water and SC lipid bilayers is estimated to be

$$K_l = K_{oct}^{0.76} = (10^{-1.38})^{0.76} = 0.0894 \quad \text{(A-7)}$$

where K_{oct} is its octanol–water partition coefficient. Substitution of eq. A-7 into the expression for P_l yields

$$D_l = P_l/K_l = (3.11 \times 10^{-11}\,cm^2s^{-1})/(0.0894) = 3.5 \times 10^{-10}cm^2s^{-1} \quad \text{(A-8)}$$

The value of D_l calculated in this manner is similar to that calculated for the transverse diffusion of water across other lipid bilayer membranes (e.g., $D = 2 \times 10^{-10}$ cm^2s^{-1}) for a tetradecane lecithin bilayer.[47] This similarity lends some credence to the transcellular permeation argument as well as the choice of K_l. The values just presented may be substituted into eq. A-1 to obtain the reported result, $t_{lag} = 2650$ s or ~44 min. One may obtain an answer within a few percent of this value from the simplified expression

$$t_{lag}^{\infty} \cong \frac{h_l h_c}{6 D_l K_{l/c}} = \frac{h_l h_c K_c}{6 D_l K_l} = \frac{h_l h_c \varphi_1}{6 P_l} \quad \text{when} \quad K_{l/c} \ll \frac{h_c}{h_l} \ll \frac{D_c}{D_l K_{l/c}} \quad \text{(A-9)}$$

Equation A-9 shows that the lag time calculation is not particularly sensitive to the partition coefficient model for the present range of parameters because the lag time varies as $1/P_l$ in this case.

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