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Partitioning and diffusion of parathion in human dermis

Rania Ibrahim, Gerald B. Kasting*

James L. Winkle College of Pharmacy, University of Cincinnati Academic Health Center, Cincinnati, OH, USA

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ABSTRACT

A previously developed method employing the use of a dialysis membrane in series with human dermis tissue mounted in side-by-side diffusion cells was utilized to observe the effects of the presence of soluble proteins in the donor compartment on the measured transport parameters of parathion. In the presence of the dialysis membrane the partition coefficient was significantly lower and the diffusion coefficient significantly higher than those determined in its absence; however, the difference was less than that previously determined for the more highly protein bound compound, diclofenac. The result suggests the dialysis membrane method is important for studying permeants that are more than about 87% bound to soluble proteins in the dermis. The results are discussed in the context of a predictive model for partitioning and transport of low molecular weight solutes in human dermis.

1. Introduction

The stratum corneum is the rate-limiting barrier to the permeation of polar and moderately lipophilic compounds, whereas, for highly lipophilic compounds this rate-limiting barrier may be dominated by the lower skin layers (Cleek and Bunge, 1993). The binding of these often highly protein-bound solutes to extravascular albumin and other soluble proteins within the dermis is thought to contribute to their transport across this layer (Cross et al., 2003; Kretsos et al., 2008; Weiss et al., 2008). Soluble proteins present in the dermis can migrate slowly through the tissue. This potential movement necessitates a careful choice of the in vitro methodology employed in the measurement of dermis transport parameters of highly protein-bound solutes (Ibrahim and Kasting, 2010). In particular, if soluble proteins migrate into the donor compartment of a side-by-side diffusion cell experiment, and the test permeant binds to these proteins, then an artificially low permeability would be obtained (Ibrahim and Kasting, 2010; Kretsos et al., 2008). In a previous study we showed that exclusion of soluble proteins from the donor compartment of side-by-side diffusion cells had a significant effect on the measured transport parameters for a highly protein-bound solute, diclofenac. The study also showed that the diffusion of the soluble proteins into the donor compartment of the cells had no effect on the measured transport parameters for a moderately protein-bound compound, DEET. The objective of the present study is to determine whether this phenomenon is important for parathion, which has a partition coefficient and protein binding affinity intermediate between DEET and diclofenac.

2. Materials and methods

2.1. Materials

Dialysis membrane (5000 Da cut-off) was purchased from Bel-Art Products (Pequannock, NJ). Unlabeled parathion (98.8%) was purchased from Sigma–Aldrich (Atlanta, GA). [Carbonyl-^{14}C]-parathion (0.8 mCi/mmol, radiochemical purity > 99%) was purchased from American Radiolabeled Chemicals (St. Louis, MO). Ultima Gold™ XR scintillation cocktail and Solvable™ were purchased from Perkin-Elmer (Boston, MA). Skin from abdominoplasty (3 donors) was obtained from Musculoskeletal Transplant Foundation (Edison, NJ). Phosphate-buffered saline (PBS), bovine serum albumin (BSA) and sodium azide were purchased from Sigma Chemicals.

* Corresponding author at: James L. Winkle College of Pharmacy, University of Cincinnati Academic Health Center, P.O. Box 670004, Cincinnati, OH 45267-0004, USA.
E-mail addresses: Gerald.Kasting@uc.edu, kastingb@ucmail.uc.edu (G.B. Kasting).

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2.2. Protein binding

Binding of ^{14}C-parathion in a 2% (w/v) BSA solution in PBS was determined by equilibrium dialysis as previously described (Ibrahim and Kasting, 2010). The 2% (w/v) level was chosen as it is the approximate average concentration of albumin in human dermis (Kretsos et al., 2008).

2.3. Dermis transport studies

Parathion transport studies in human dermis were conducted and analyzed as previously described (Ibrahim and Kasting, 2010). Briefly, skin samples from abdominoplasties were heat separated (Kligman and Christophers, 1963) to isolate the dermis. The dermis was mounted in series with a dialysis membrane (donor side) in water-jacketed, side-by-side diffusion cells maintained at 37 °C. The donor and receptor compartments were filled with PBS and 2% BSA–PBS solution respectively and allowed to equilibrate for 24 h. BSA was added to the receptor solution to maintain endogenous levels of albumin in the dermis (Kretsos et al., 2008) and to ensure sink conditions in the receptor solution for highly lipophilic solutes. Unlike the previously described studies (Ibrahim and Kasting, 2010), both the donor and receptor compartments were then emptied and replenished with fresh solutions. The new donor solution contained 3.62 μg/mL and 0.1 μCi/mL of ^{14}C-parathion in PBS. This procedure was chosen due to inadequate mixing when ^{14}C-parathion was spiked directly into the donor compartment. Studies for Donor 1 were carried out for 6 h and Donors 2 and 3 studies were carried out for 32–48 h to ensure steady-state diffusion. The run time difference was due to the considerable difference in thickness of the samples. At the end of the experiment each dermis sample was dissolved in Solvable™ (1 mL) and analyzed by LSC. Additional studies were conducted using dermis samples mounted without dialysis membrane and the results were compared.

The cumulative amount of solute passing through unit area of membrane $M(t)$ was plotted versus time. The steady state flux J_{ss} was calculated as the slope of the linear portion of the graph, and the time lag T_L was calculated as the intercept of the regression line on the time axis. The permeability coefficient k_p and total diffusive resistance R_{tot} were calculated according to Eq. (1),

$$k_p = \frac{1}{R_{tot}} = \frac{J_{ss}}{\Delta C} \approx \frac{J_{ss}}{C_d} \tag{1}$$

where C_d is the donor solution concentration. The approximation $\Delta C \cong C_d$ was justified since the ratio of unbound permeant concentration in the receptor solution to that in the donor solution was always less than 7%.

The diffusive resistance of the dermis R_{de} to parathion was calculated according to Eq. (2)

$$R_{de} = R_{tot} - R_{Dial} - (R_1 + R_2) \tag{2}$$

where R_{Dial} is the resistance of the dialysis membrane and in the absence of the dialysis membrane was set equal to zero. R_1 and R_2 are the resistances of the aqueous boundary layers in the donor and receptor compartments, respectively,

$$R_1 = \frac{h_{ABL}}{D_{aq1}} \tag{3}$$

$$R_2 = f_u \frac{h_{ABL}}{D_{eff}} \tag{4}$$

In Eqs. (3) and (4) h_{ABL} is the thickness of the aqueous boundary layer (0.017 cm) (Ibrahim and Kasting, 2010), D_{aq1} is the diffusivity of unbound parathion in water, f_u is the fraction unbound, and D_{eff} is the effective diffusivity of parathion in the receptor solution (Ibrahim and Kasting, 2010),

$$D_{eff} = f_u D_{aq2} + (1 - f_u) D_{bound} \tag{5}$$

Here D_{bound} is the diffusivity of albumin in the boundary layer, taken to be equal to the diffusivity of BSA in water at 37 °C, 9.29×10^{-7} cm^2/s (Fardet et al., 1998). The value of D_{aqi} for parathion at 37 °C was estimated according to the Wilke–Chang relationship (Poling et al., 2001):

$$D_{aqi} \ (\text{cm}^2\,\text{s}^{-1}) = \frac{7.4 \times 10^{-8} (\phi M)^{1/2} T}{\eta_i V_A^{0.6}} \tag{6}$$

where $\phi = 2.26$, $M = 18.01$ g mol^{-1} and $T = 310.15$ K. The value of η_i was taken to be 0.6915 cP in the donor solution (Eq. (3), $i = 1$) and 0.7468 cP in the receptor solution (Eqs. (4) and (5), $i = 2$) based on viscosity estimates for albumin solutions discussed later. The values of ϕ and η_i represent slight improvements over those chosen by Ibrahim and Kasting (2010), but they do not significantly change the earlier results. It is noteworthy that the value 2.26 for the association factor for water stems from work by Hayduk and Laudie (1974) that is not incorporated in some modern references, e.g. Poling et al. (2001). The molar volume at the boiling point, V_A, was estimated using Schroeder's Method (Poling et al., 2001) to be 279.5 cm^3/mol. Eq. (6) then yielded D_{aqi} values of 7.23×10^{-6} cm^2/s and 6.69×10^{-6} cm^2/s for parathion in the donor and receptor solutions, respectively. The value $R_{Dial} = 24{,}700$ s/cm for parathion was then estimated by interpolating between the values of R_{Dial} for DEET (21,200 s/cm) and diclofenac (24,900 s/cm) based on the assumption that $R_{Dial} \propto {D_{aq1}}^{-1}$ (Ibrahim and Kasting, 2010). Here the donor solution value for D_{aq} was used since albumin is excluded from the membrane. In making this estimate we recalculated D_{aq1} for DEET (7.94×10^{-6} cm^2/s) and diclofenac (7.20×10^{-6} cm^2/s) using the updated values of ϕ and η_i. Insertion of these values into Eqs. (3)–(5) led to the result that $R_1 = 2351$ s/cm, $R_2 = 1285$ s/cm and $D_{eff} = 1.77 \times 10^{-6}$ cm^2/s.

The product of dermis diffusivity D_{de} and partition coefficient K_{de}, often termed permeability P_{de}, was calculated from R_{de} and the thickness h_{de} of each sample according to Eq. (7):

$$P_{de} = D_{de} K_{de} = \frac{h_{de}}{R_{de}} \tag{7}$$

The value of K_{de} was determined from the average concentration measured in the dermis tissue sample $\bar{C}_{de}$ after correction for series resistances; thus (Ibrahim and Kasting, 2010)

$$K_{de} = \frac{2\overline{C_{de}}}{C_d} \left(\frac{R_{tot}}{R_{tot} - R_1 - R_{Dial} + R_2} \right) \tag{8}$$

The dermis diffusivity D_{de} was then calculated as

$$D_{de} = \frac{P_{de}}{K_{de}} \tag{9}$$

As a comparison, D_{de} was also estimated from the time lag T_L according Eq. (10), which assumes the time lag is dominated by the dermis.

$$D_{de} = \frac{h_{de}^2}{6 T_L} \tag{10}$$

The validity of assuming symmetrical boundary layers in the donor and receptor solutions (Eqs. (3) and (4)) and a time lag dominated by the dermis (Eq. (10)) is discussed later.

2.4. Statistical analysis

Results were calculated individually for each diffusion cell, and then averaged to obtain a mean and standard error. Transport and

Table 1
Physical properties of parathion.

Property	Units	Value
MW	Da	291.26
V_A	cm^3/mol	279.5[a]
log K_{oct}	–	3.83[b]
S_w[c]	g/L	0.011[b]
f_u[d]	–	0.134 ± 0.005[e]

[a] Schroeder's method (Poling et al., 2001). A value of 20.5 cm^3/mol was used for phosphorus.
[b] US_EPA (2009).
[c] Water solubility at 20 °C.
[d] Fraction unbound in a 2% (w/v) BSA solution.
[e] Mean ± SE (n = 5).

partitioning parameters were compared via two-way ANOVA using donor and presence or absence of a dialysis membrane as blocking variables. The pairwise comparison test used was Holm–Sidak; values of $p < 0.05$ were considered to be significant. For comparisons involving only two groups, a Student's t-test was employed. All tests were conducted using SigmaStat version 3.10 (SYSTAT, Chicago, IL).

3. Results

Physical properties of parathion are shown in Table 1. The fraction unbound in 2% albumin solution was determined to be 0.134 ± 0.005 (n = 5). The results for the dermis transport studies are shown in Table 2 and Fig. 1. Data collected after 32 h for Donors 2 and 3 (not shown) were found to depart from linearity and were therefore not included in the analysis. Transport and partitioning parameters calculated from the data in the presence and absence of dialysis membrane showed significant differences between the dermis diffusivity D_{de} (p = 0.019) and partition coefficient K_{de} ($p < 0.001$). A lower K_{de} and a higher D_{de} was observed in the absence of the dialysis membrane. Despite the lower K_{de} in the absence of the dialysis membrane, a higher permeation of parathion was observed (Fig. 1). Diffusivities calculated from the time lag (Eq. (10)) were generally higher than those calculated from the permeability data (Eq. (9)).

4. Discussion

The analysis described by Eqs. (1)–(10) involves several assumptions that were briefly set forward in Ibrahim and Kasting (2010) and are now discussed further. It is assumed that transport in the solutions bounding the membrane can be adequately described by film theory rather than the more elaborate boundary layer theory (Cussler, 1997). This assumption has been found to be adequate for side-by-side diffusion cells of a similar design (Tojo et al., 1985). The widths of the aqueous boundary layers in the donor and receptor solutions, h_{ABL}, are assumed to be equal. This is reasonable since the cells are symmetrical and 2% BSA imparts little additional viscosity to the PBS solution. The effect may be estimated as follows: The intrinsic viscosity of BSA ($\lim_{c\to 0}\eta_{sp}/c$) is about 4 mL/g, where $\eta_{sp} = (\eta - \eta_0)/\eta_0$ is the fractional viscosity increase imparted by a concentration of c g/mL; furthermore the concentration dependence of BSA aqueous solution viscosity is linear for c up to 0.065 g/mL or 6.5% (w/v) (Friedli, 1996). Thus addition of 2% (w/v) BSA to water increases its viscosity η by about 8%, i.e. η_{sp} = 0.08. Hence, the viscosity of a 2% (w/v) BSA solution at 37 °C is about 0.75 cP, slightly higher than that of water (0.69 cP). Following the analysis of Tojo et al. (1985), h_{ABL} scales as $\eta^{0.70}$; therefore an 8% increase in η would increase h_{ABL} by 5–6%. This change is well within the uncertainty of the analysis. Finally, Eq. (10) assumes the time lag (t_L) to be fully determined by diffusion in the dermis. Application of multilaminate time lag models (Ash et al., 1965; Sinko,

Table 2
Transport and Partitioning Parameters and Data Associated With the Parathion Dermis Transport Studies (Mean ± SE).

Treatment	Donor (n)[a]	$k_p \times 10^5$ (cm/s)	$R_{tot} \times 10^{-5}$ (s/cm)	$R_{de} \times 10^{-5}$ (s/cm)	$P_{de} \times 10^6$ (cm^2/s)	K_{de}	$D_{de} \times 10^6$ (cm^2/s)		h (cm)	T_L (h)
							Eq. (9)	Eq. (10)		
Dialysis + dermis	1 (12)	1.99 ± 0.10	0.52 ± 0.03	0.24 ± 0.03	7.55 ± 0.44	5.56 ± 0.27	1.17 ± 0.07	1.17 ± 0.08	0.17 ± 0.01	1.17 ± 0.11
	2 (7)	0.95 ± 0.08	1.11 ± 0.11	0.83 ± 0.11	3.68 ± 0.37	5.98 ± 0.64	0.66 ± 0.13	0.81 ± 0.07	0.28 ± 0.02	4.75 ± 0.49
	3 (7)	0.69 ± 0.08	1.62 ± 0.25	1.35 ± 0.25	2.63 ± 0.31	6.46 ± 0.71	0.45 ± 0.09	1.29 ± 0.18	0.31 ± 0.02	3.75 ± 0.41
Mean ± SE (all replicates)					5.18 ± 0.50	5.91 ± 0.28	0.83 ± 0.08	1.10 ± 0.07		
Dermis only	1 (15)	4.26 ± 0.49	0.28 ± 0.03	0.24 ± 0.03	7.79 ± 0.70	2.88 ± 0.20	2.58 ± 0.42	4.21 ± 0.67	0.17 ± 0.01	0.42 ± 0.07
	2 (10)	1.17 ± 0.16	1.04 ± 0.18	1.00 ± 0.18	3.30 ± 0.39	5.26 ± 0.79	0.94 ± 0.30	2.19 ± 0.19	0.28 ± 0.01	1.73 ± 0.19
	3 (9)	1.07 ± 0.24	1.49 ± 0.34	1.45 ± 0.34	3.32 ± 0.60	3.14 ± 0.97	2.66 ± 0.94	3.52 ± 1.08	0.33 ± 0.02	2.08 ± 0.27
Mean ± SE (all replicates)					5.28 ± 0.53	3.65 ± 0.39[†] ($p < 0.001$)	2.12 ± 0.34[†] ($p \le 0.05$)	3.43 ± 0.43[†]		

[a] Donor (total no. of replicates).
[†] Statistical significance between treatments.

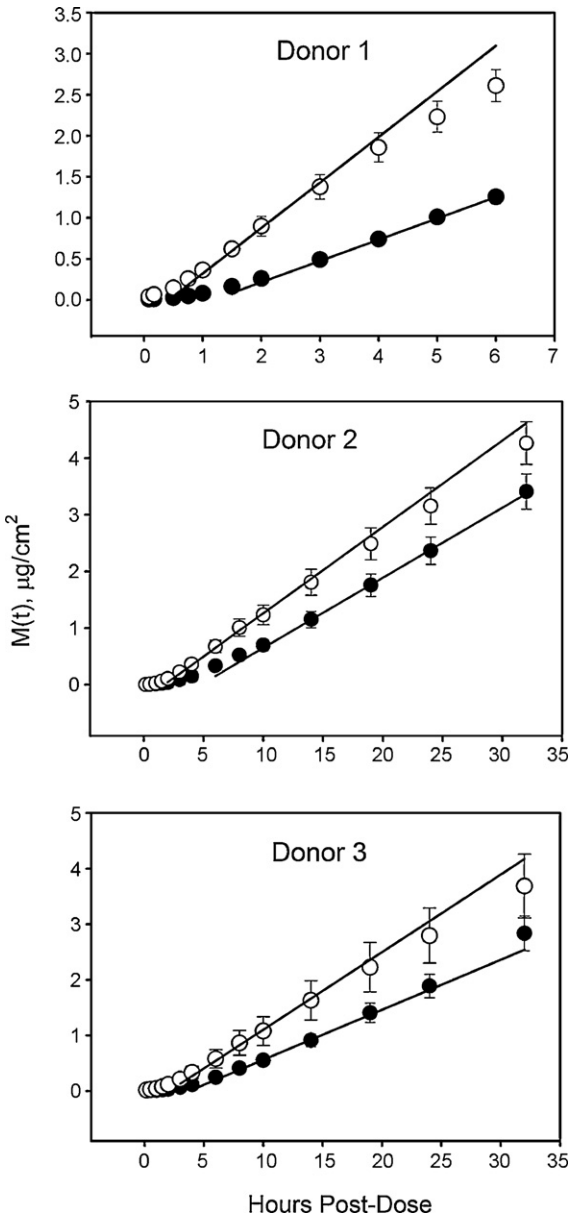

Fig. 1. Results of skin dermis permeation studies for donors 1, 2 and 3 in the presence (●) and absence (○) of a dialysis membrane placed between the dermis and donor solution. The donor solutions contained ^{14}C-parathion dissolved in PBS and the receptor solutions contained PBS + 2% BSA. The solid lines were calculated as $M(t) = k_p C_d(t - T_L)$ using the average values of k_p and T_L in Table 2 and C_d = 3.62 μg/mL.

2011) to the data in Table 2 suggests this approximation cannot be justified. The dialysis membrane in particular is expected to contribute to the time lag since its diffusive resistance R_{Dial} is an appreciable fraction of the dermis resistance and its effective partition coefficient K_{Dial} is very low. This effect may be estimated as follows: The thickness of this membrane was about 20 μm and R_{Dial} was estimated above to be 24,700 s/cm. Assuming an aqueous diffusivity within the pores of 7.23×10^{-6} cm^2/s yields an effective K_{Dial} value of 0.0112. Insertion of these values plus average values for the dermis (Table 2, Row 4) into the two-layer time lag formula given in Sinko (2011) leads to the prediction that the dialysis membrane should increase the time lag by 67% with respect to a dermis-only experiment. Examination of Table 2 shows that the actual impact of the dialysis membrane on the observed time lag is somewhat larger than this. However quantitative agreement between the time lags

Table 3
Transport and partitioning parameters in human dermis for selected permeants (mean ± SE).

Compound	$D_{de} \times 10^6$ (cm^2/s)	$K_{de/pH7.4}$	$P_{de} \times 10^6$ (cm^2/s)
DEET			
Observed[a,b]	4.59 ± 1.41	0.83 ± 0.35	3.33 ± 0.86
Kretsos et al. (2008)	0.90	1.51	1.36
Dancik et al. (2012)	0.96	1.51	1.44
Eqs. (11)–(14)	1.41	1.51	2.13
Diclofenac			
Observed[a,b]	0.57 ± 0.06	7.56 ± 0.67	3.95 ± 0.31
Kretsos et al. (2008)	0.196	5.22	1.02
Dancik et al. (2012)	0.284	5.22	1.48
Eqs. (11)–(14)	1.02	5.22	5.34
Parathion			
Observed[b,c]	0.83 ± 0.08	5.91 ± 0.28	5.18 ± 0.50
Kretsos et al. (2008)	0.175	5.90	1.03
Dancik et al. (2012)	0.196	5.90	1.16
Eqs. (11)–(14)	0.372	5.90	2.20
Glucose			
Observed[d]	2.64 ± 0.42	0.65 ± 0.09	1.36 ± 0.22
Kretsos et al. (2008)	2.36	0.61	1.41
Dancik et al. (2012)	2.36	0.61	1.41
Eqs. (11)–(14)	2.36	0.61	1.41

[a] Ibrahim and Kasting (2010).
[b] Average of all dermis + dialysis measurements.
[c] Table 2.
[d] Khalil et al. (2006).

predicted from multilaminate slab models and those observed is poor. Hence, neither Eq. (10) nor multilaminate variations thereof can be recommended and diffusivity of the permeant in the dermis should be calculated from Eq. (9).

Kretsos et al. (2008) noted that the conventional in vitro permeation and partition measurements of solutes in the dermis could be confounded by diffusion of soluble proteins from the tissue. This hypothesis was confirmed in a previous study from this laboratory employing DEET and diclofenac (Ibrahim and Kasting, 2010). Whereas results for DEET (f_u = 0.189 ± 0.004) were not affected by the insertion of a barrier membrane between the donor solution and dermis, results for diclofenac (f_u = 0.040 ± 0.005) were sensitive to this change. In the case of diclofenac, contrary to what one would expect, a higher permeation was observed in the absence of the dialysis membrane. This was attributed to the presence of soluble proteins in the donor compartment (Ibrahim and Kasting, 2010). The objective of this study was to test the new methodology with parathion, a compound whose protein-binding affinity as expressed in terms of fraction unbound (f_u = 0.134 ± 0.005) lies between DEET and diclofenac. We had to modify the "control" methodology due to the low water solubility of parathion, which led to inadequate mixing when the donor solution was spiked with labeled solute immediately prior to the study as in the previous experiments. In the present study, following the equilibration period, the PBS solution was removed and replenished with a donor solution of radiolabeled parathion which had been agitated overnight. Thus, a substantial amount of the diffused soluble protein was removed. Yet, the results were still impacted by the presence or absence of dialysis membrane. Significant differences in D_{de} and K_{de} were obtained, although smaller than those observed for diclofenac. We conclude that compounds that are more than about 87% bound to soluble proteins in the dermis should be tested using the new methodology.

It is of interest to compare the results of these experiments with the predictions based on current models of dermis permeability. A limited comparison, using data and models generated by our research group, is shown in Table 3. The relationships employed in these calculations may be found in Kretsos et al. (2008) or Dancik et al. (2012) and the permeant physical properties may be found in the original references or in Table 1 (for parathion). The (Kretsos

et al., 2008) model assumes permeant bound to albumin to be immobile. Dancik et al. (2012) impute some mobility to bound permeant, assigning it a diffusivity $D_{\text{bound}}^{\text{tissue}}$ of 1×10^{-7} cm^2/s in the albumin-accessible region of the dermis only. The (Kretsos et al., 2008) calculation works effectively to predict K_{de}; consequently it has not been changed. It also gave a satisfactory prediction for D_{de} for glucose (which does not bind to proteins), but both Kretsos et al. (2008) and Dancik et al. (2012) substantially underestimate D_{de} for the other three permeants. Since $P_{\text{de}} = D_{\text{de}}K_{\text{de}}$, dermis permeability is also underestimated. In order to correct this we propose the following modification to the calculation scheme presented in Dancik et al. (2012):

$$\text{BF} = 0.68 + \frac{0.32}{f_{\text{u}}} + 0.001 f_{\text{non}} K_{\text{oct}} \tag{11}$$

$$K_{\text{de/pH7.4}} = 0.6 \times \text{BF} \tag{12}$$

$$\log D_{\text{free}} = -4.15 - 0.655 \log \text{MW} \tag{13}$$

$$D_{\text{de}} = |f_{\text{u}} D_{\text{free}} + (1 - f_{\text{u}}) D_{\text{bound}}^{\text{tissue}}| / (f_{\text{u}} BF);$$
$$D_{\text{bound}}^{\text{tissue}} = 3 \times 10^{-7}\ \text{cm}^2\text{s}^{-1} \tag{14}$$

In Eqs. (11)–(14), BF is a binding factor associated with permeant binding to mobile proteins as well as partitioning into immobile lipids, f_{u} is the unbound fraction, f_{non} is the fraction nonionized, K_{oct} is octanol/water partition coefficient and MW is molecular weight. K_{de} has been expressed relative to a pH 7.4 buffer to coincide with the experiments described herein. In order to calculate its value with respect to the nonionized concentration in water, the result from Eq. (12) should be divided by f_{non} (Kretsos et al., 2008). The value of $D_{\text{bound}}^{\text{tissue}}$ has been increased three-fold with respect to Dancik et al. (2012), and it applies broadly across the tissue. The net change is a ten-fold larger contribution of bound permeant to D_{de}. In addition to providing a better match to the data in Table 3, a rationale for this change may be drawn from the work of Jain and coworkers (Chary and Jain, 1989; Nugent and Jain, 1984). Using two different optical methods, FITC-labeled albumin and an in vivo rabbit ear "sac" model, these workers measured albumin diffusivities in tissue ranging from 0.11×10^{-7} cm^2/s to 5.8×10^{-7} cm^2/s. The former value was obtained with a relaxation method (Nugent and Jain, 1984), the latter with Fluorescence Recovery After Photobleaching (Chary and Jain, 1989). The investigators argued that the relaxation method measures diffusion in the gel phase of the dermis, whereas FRAP is sensitive to diffusion in the fluid phase. Notably FRAP diffusivity of FITC-albumin fell to 1.7×10^{-7} cm^2/s in sacrificed rabbits. The investigators proposed that the fluid channels collapsed in the dead animals, leading to lower diffusivities. Considering these results it seems reasonable to propose the value $D_{\text{bound}}^{\text{tissue}} = 3 \times 10^{-7}$ cm s^{-1} in Eq. (14) for permeants in excised human dermis that primarily bind to albumin. It is furthermore possible that a larger value could apply to human dermis in vivo due to the presence of smaller binding proteins and (potentially) more open fluid channels.

The use of these values is as follows: Eqs. (11)–(14) summarize the results of a microscopic model for dermis partitioning and transport (Kretsos et al., 2008) that leads to a macroscopic value of the partition coefficient K_{de} and an effective diffusivity D_{de} that, when multiplied together, yield an estimate for dermis permeability P_{de} that may be used in a homogenized transport model in which each skin layer is represented as a uniform slab (Dancik et al., 2012). For ionizable solutes care must be taken to choose an appropriate reference state for K_{de} as described above.

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