*Pharmaceutical Research, Vol. 5, No. 9, 1988*

*Research Article*

# Heterogeneity Effects on Permeability–Partition Coefficient Relationships in Human Stratum Corneum

**Bradley D. Anderson,[1,3] William I. Higuchi,[1] and Prakash V. Raykar[2]**

*Received October 14, 1987; accepted March 9, 1988*

The relationship between the permeability of solutes undergoing transport via the lipid pathway of the stratum corneum and the degree to which the same solutes partition into the stratum corneum has been explored by measuring the permeability coefficients and stratum corneum/water partition coefficients of a series of hydrocortisone esters varying in lipophilicity. Isolated human stratum corneum, used in both the permeability and the uptake experiments, was shown to resemble full-thickness skin in its overall resistance and selectivity to solute structure. As with full-thickness skin, delipidization destroys the barrier properties of isolated stratum corneum. Although a linear relationship is frequently assumed to exist between permeability coefficients and membrane/water partition coefficients, a log–log plot of permeability coefficients versus the intrinsic stratum corneum/water partition coefficients for the series of hydrocortisone esters studied is distinctly nonlinear. This nonlinearity arises from the fact that the transport of these solutes is rate limited by a lipid pathway in the stratum corneum, while uptake reflects both lipid and protein domains. From the relative permeability coefficients of 21-esters of hydrocortisone varying in acyl-chain structure, group contributions to the free energy of transfer of solute into the rate-limiting barrier microenvironment of the stratum corneum lipid pathway are calculated for a variety of functional groups including the $-CH_2-$, $-CONH_2$, $-CON(CH_3)_2$, $-COOCH_3$, $-COOH$, and $-OH$ groups. These are compared to contributions to the free energies of transfer obtained for the same functional groups in octanol/water, heptane/water, and stratum corneum/water partitioning experiments. The group contributions to transport for polar, hydrogen-bonding functional groups are similar to the values obtained from octanol/water partition coefficients. This similarity suggests that complete loss of hydrogen bonding does not occur in the transition state for passive diffusion via the lipid pathway.

**KEY WORDS:** stratum corneum; partition coefficients; hydrocortisone esters; lipids; proteins, uptake; transport; functional-group contributions.

## INTRODUCTION

The stratum corneum, the keratinized outer layer of human skin, has been widely recognized as a primary transport barrier for most solutes of pharmaceutical interest (1–4). While no clear mechanistic picture of the nature of this barrier has emerged, both polar and lipid transport pathways have been suggested (5). The principle evidence for the existence of parallel polar and lipid pathways comes from solute permeability–lipophilicity relationships (4,6,7). Highly hydrophilic solutes are believed to permeate via the so-called "polar" or "pore" route due to the fact that increasing solute lipophilicity of very polar solutes has a negligible effect on permeability coefficients. A "lipid" pathway is invoked to account for the fact that the permeability coefficients of solutes having an intermediate polarity increase with increasing solute lipophilicity.

Although the existence of distinct hydrophilic and lipophilic transport pathways has been suggested for many years, morphologic evidence for segregated lipid and protein domains is more recent (8,9). It is now clear from freeze-fracture, thin-section, histochemical, and cytochemical studies that the stratum corneum lipids are located in intercellular spaces, while the protein domain, which consists largely of cross-linked keratin, resides intracellularly (10–13). Recent studies in this laboratory have further shown that the protein and lipid domains in human stratum corneum exhibit different selectivities with respect to solute uptake, resulting in a change of uptake mechanism with increasing solute lipophilicity (14). Whether or not these two morphologically distinct domains represent the polar and lipid transport pathways is unknown.

The primary objectives of this work were (a) to test the assumption of a linear relationship between solute permeabilities and stratum corneum/water partition coefficients for solutes undergoing transport via the lipid pathway and (b) to generate group contributions to the transfer of hydrocortisone esters from water to the rate-limiting barrier microenvironment in the stratum corneum for a variety of functional groups of varying polarity. In pursuit of these

[1] Department of Pharmaceutics, College of Pharmacy, University of Utah, Salt Lake City, Utah 84108.

[2] In partial fulfillment for the Ph.D. degree in pharmaceutics, College of Pharmacy, University of Utah.

[3] To whom correspondence should be addressed.

0724-8741/88/0900-0566$06.00/0 

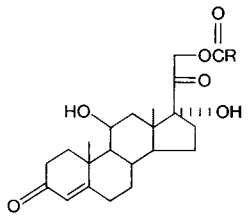
aims, methods for monitoring solute transport through intact sheets of human stratum corneum isolated from full-thickness skin were developed.

## MATERIALS AND METHODS

Methods for the synthesis of hydrocortisone esters varying in lipophilicity (Scheme I) and the high-performance liquid chromatographic (HPLC) methods employed in their analyses were reported previously (14). Fluocinonide used in these studies was provided by Syntex Research, Palo Alto, Calif. Other reagents were of analytical reagent grade obtained from commercial sources and used without further purification.

*Preparation of Human Stratum Corneum.* Full-thickness human skin was obtained from cadavers at autopsy or from elective abdominoplastic surgery (Department of Dermatology, School of Medicine, University of Utah, Salt Lake City). The source of skin with respect to body region, age, sex, etc., was not controlled but such information was recorded for each sample. Skin samples were stored 7–10 days in tissue culture medium (RPMI-1640, Flow Laboratories, McLean, Va.) with 5% bovine serum at 4°C prior to isolation of the stratum corneum. Two methods of separating the epidermis from the dermis were employed—dry heat treatment [55–58°C, 45 sec (15)] or EDTA treatment [0.75% EDTA, 2 hr, 37°C (14,16)]—followed by trypsin treatment to digest the viable epidermis as described previously (14). The transport properties of stratum corneum isolated by the two methods did not differ significantly. Delipidization of stratum corneum was by 2:1 chloroform–methanol extraction (14,17,18).

*Stratum Corneum/Water and Solvent/Water Partition Coefficients.* The measurement of stratum corneum/water partition coefficients and octanol/water partition coefficients of the hydrocortisone esters of interest was described previously (14). Heptane/water partition coefficients were determined by the shake flask method (19) in a similar manner to the octanol/water partition coefficients. Briefly, 10 ml of saturated aqueous solution of solute (buffered at pH 4) was combined in a separatory funnel with 250 ml of heptane and the separatory funnel was placed in a water bath at 37°C. Either the sample was shaken intermittently or the heptane phase was stirred continuously for at least 25 hr. After equilibration, known volumes of the heptane and water phases were analyzed for solute.




1a–k

| COMPOUND | R |
|---|---|
| 1a | $-CH_2CH_2CONH_2$ |
| 1b | $-CH_2CH_2CON(CH_3)_2$ |
| 1c | $-CH_2CH_2COOCH_3$ |
| 1d | $-CH_2CH_2COOH$ |
| 1e | $-(CH_2)_4CH_2COOH$ |
| 1f | $-(CH_2)_4CH_2CONH_2$ |
| 1g | $-(CH_2)_4CH_2OH$ |
| 1h | $-CH_2CH_3$ |
| 1i | $-(CH_2)_4CH_2COOCH_3$ |
| 1j | $-(CH_2)_4CH_3$ |
| 1k | $-(CH_2)_6CH_3$ |

Scheme I

*Transport Studies.* Transport experiments were carried out in two chamber diffusion cells (20). Circular pieces of full-thickness or isolated stratum corneum were placed securely between the two halves of the cells and held by a ball-point clamp to give an exposed surface area of 0.65 $cm^2$. The assembled cell was immersed in a water bath maintained at 37°C and both donor and receiver chambers were constantly stirred at 150 rpm by Teflon paddles attached to direct drive motors (Model CA, Hurst Manufacturing Corporation, Princeton, Ind.). Prior to beginning the diffusion experiments, the isolated stratum corneum samples were allowed to hydrate *in situ* for 24 hr, after which both chambers were emptied and the receiver side was filled with 2 ml receiver solution. The transport run was initiated by charging the donor chamber with the appropriate donor solution. At various times portions of the receiver solution were sampled and the entire receiver side was rinsed and refilled with fresh solution preequilibrated to 37°C. The donor concentration was maintained constant by replacing the donor solution with fresh solution at every time point (21).

Donor solutions of various hydrocortisone derivatives were buffered to pH 4, the pH of optimum ester stability. Saturated solutions were prepared by placing an excess of the desired compound in pH 4 succinate buffer (22) and shaking at 25°C for 72 hr. The suspensions were filtered through a 0.45-μm filter (Acrodisc-CR, Gelman Sciences Inc., Ann Arbor, Mich.). The first three successive 10-ml portions of the filtrate were discarded to minimize loss of solute due to adsorption to the filter. The final donor solute concentrations were analyzed by HPLC. The receiver solutions were also buffered to pH 4. Due to the very low aqueous solubility of hydrocortisone octanoate (**1k**), a suspension of this solute was used to maintain saturation in the donor side.

The physical integrity of the stratum corneum membranes was assessed during some experiments by spiking the donor side with a known specific activity of $^{14}C$-sucrose and monitoring its transport. Leaks in the stratum corneum were readily detected by this method. In addition, the permeability of hydrocortisone propionate was determined simultaneously with the solute of interest in every diffusion experiment to normalize for skin sample-to-sample variability.

## RESULTS AND DISCUSSION

### Permeant Selection

Knowledge of the influence of solute molecular structure on skin penetrability can serve two purposes. First, such information is essential in identifying the transport mechanism involved and characterizing the solvent nature of the barrier microenvironment. Second, a quantitative data base relating chemical structure to skin permeability would be most valuable from a practical standpoint for use in predicting the permeation rate of a new drug candidate or

as a guide in the design of molecules with enhanced penetration.

Permeability coefficients ($k_p$) of solutes transported via the lipid pathway are generally assumed to be directly proportional to the stratum corneum partition coefficients, $K_m$, of the same solutes as expressed by the following relationship:

$$k_p = \frac{K_m D_m}{\lambda} \quad (1)$$

$D_m$ is the diffusion coefficient of solute in the membrane and $\lambda$ is the stratum corneum thickness. However, this relationship assumes that the membrane is homogeneous. Considerable evidence has been generated to date indicating that morphologically distinct domains exist within the stratum corneum, with the transport pathway for most solutes of pharmaceutical interest residing within the intercellular lipid domains of the stratum corneum (8–13). Therefore, the extent to which Eq. (1) is applicable to transport via the lipid pathway of stratum corneum, given the heterogeneity of this membrane, must be ascertained.

To gain further insight into the nature of the stratum corneum lipid pathway, we have determined the relative rates of diffusion through isolated human stratum corneum of a series of 21-esters of hydrocortisone differing in terminal substitution and/or methylene-chain length (Scheme I). The compounds used in this study were selected because (a) hydrocortisone esters in this lipophilicity range have previously been shown to undergo rate-limited transport via the lipid pathway of stratum corneum (5,23), the pathway of interest in this investigation, and (b) a variety of functional group contributions to the transport process could be examined within this series.

The quest for a model bulk solvent which mimics the solvent nature of the stratum corneum barrier microenvironment has been a unifying theme of structure–uptake and permeability investigations (2,6,14,23,24). Previous studies of the influence of molecular structure on skin permeation rates have generally focused on the methylene-group contribution (2,23,24) obtained from comparisons of relative permeability coefficients within homologous series. The values typically obtained in skin transport studies for the methylene-group contribution (see Ref. 23 and Table IV) are small compared to the corresponding values observed for hydrocarbon/water or octanol/water partitioning, suggesting that the stratum corneum barrier exhibits a selectivity to chain length similar to a relatively polar bulk solvent. However, the methylene group is not a very sensitive probe of bulk solvent polarities compared to other functional groups such as $-CONH_2$, $-OH$, etc. (25). Moreover, the transfer process from water to the rate-limiting microenvironment of the stratum corneum may involve transfer into the hydrocarbon region of lipid bilayers. Partitioning of an alkyl group into lipid bilayers would be relatively less favorable than into a bulk hydrocarbon solvent due to the highly ordered nature of bilayer chains (26,27) and the corresponding negative entropic contribution associated with the insertion of such a group into the bilayer region (28,29). Thus, the small methylene-group increments observed in skin transport studies may not reflect the polarity of the barrier microenvironment but, rather, its degree of order. Examining a wide variety of functional-group contributions might be useful in differentiating between a highly ordered and a relatively polar environment since the effects of bilayer chain ordering would not be expected to be functional group specific. Therefore in this work we have studied the effect of various functional groups, in addition to the methylene group, on solute transport and uptake.

### Barrier Properties of Isolated Human Stratum Corneum Before and After Delipidization

The use of isolated human stratum corneum in both transport and partition coefficient studies, in contrast to epidermis or full-thickness human skin, offers several advantages. First, the stratum corneum is rate limiting for most compounds of pharmaceutical interest, and studies of this membrane in isolation provide information without contribution to the barrier from the dermis and epidermis. Second, elucidation of the relationship between permeability coefficients and stratum/corneum partition coefficients requires knowledge of the stratum corneum/water partition coefficients. Full-thickness skin/water partition coefficients would have to be corrected for uptake into epidermal and dermal layers. A disadvantage of the use of human stratum corneum in permeability studies is the increased difficulty of obtaining leak-free membranes.

Figure 1 shows results of a typical transport experiment for which the permeability coefficients (cm/hr) of hydrocortisone propionate (**1h**), hydrocortisone methylsuccinate (**1c**), and sucrose are plotted versus time at 37°C. Permeability coefficients are typically estimated from the slopes of the linear portions of plots of cumulative amount transported versus time. Although the slopes of the linear portions of such plots are proportional to the permeability coefficients, experimental errors at individual sample points may be carried across the entire data set. The method of calculating permeability coefficients at every time interval by replacing the entire receiver chamber with fresh solution, as shown in Fig. 1, mitigates this problem (21).

The permeability coefficients of various solutes in full-thickness skin and isolated stratum corneum are listed in Table I. The similarity of the values in the two membranes illustrates that the resistance of full-thickness skin is re-

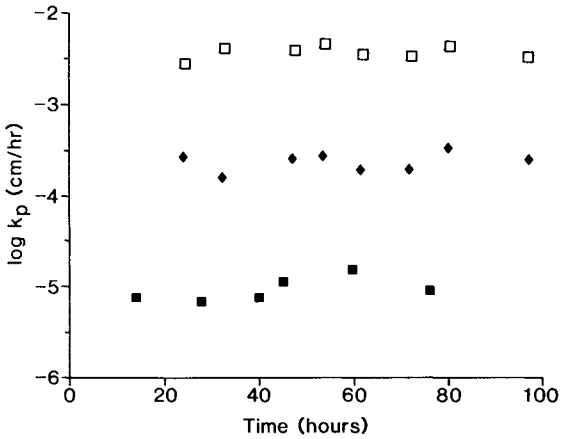



**Fig. 1.** Logarithms of permeability coefficients in human stratum corneum at 37°C obtained at various time intervals for hydrocortisone propionate (□), hydrocortisone methylsuccinate (◆), and sucrose (■).

Table I. Permeability Coefficients, $k_p$ (cm/hr ± % SD), of Various Solutes Through Full-Thickness Skin and Untreated and Delipidized Stratum Corneum at 37°C

| Compound[a] | Full-thickness skin | Isolated stratum corneum | |
|---|---|---|---|
| | | Untreated | Delipidized |
| **1h** | $3.3 \times 10^{-3}$ (±21%) | $5.2 \times 10^{-3}$ (±35%) | $1.3 \times 10^{-1}$ (±12%) |
| **1j** | — | $1.0 \times 10^{-2}$ (±4%) | $1.2 \times 10^{-1}$ (±8%) |
| Fluocinonide | $1.7 \times 10^{-3}$ (±8%) | $1.7 \times 10^{-3}$ (±12%) | — |
| Sucrose | $5.2 \times 10^{-6}$ (±21%) | $9.4 \times 10^{-6}$ (±33%) | $2.2 \times 10^{-1}$ (±8%) |

[a] **1h**, hydrocortisone propionate; **1j**, hydrocortisone hexanoate.

tained in isolated stratum corneum. Differences of approximately three orders of magnitude in permeability coefficients, depending on the solute lipophilicity, further indicate that the selectivity of the isolated stratum corneum resembles that of full-thickness skin. A very low permeability value is observed for sucrose, consistent with its low lipophilicity, thus establishing the physical integrity of the isolated stratum corneum membrane.

Studies in hairless mouse skin (30,31) have shown that permeation is enhanced with extended hydration. This is believed to be due to deterioration of the barrier (31). It is interesting to note that, with isolated human stratum corneum, no significant increases occur with over 100 hr (Fig. 1) of hydration, illustrating what may be a major difference between the human and the hairless mouse skin.

If lipids in the intercellular spaces of the stratum corneum constitute the barrier to transport, removal of lipids (delipidization) should result in the loss of barrier resistance and selectivity. In measurements of alcohol permeabilities, Scheuplein and Ross have shown that after lipid extraction (a) permeability constants increase markedly, (b) the permeabilities of similarly sized polar and nonpolar molecules become similar, and (c) the activation energy for diffusion decreases to a value consistent with aqueous diffusion (18). The data in Table I confirm that the barrier resistance and selectivity are lost upon lipid extraction. Delipidization resulted in a substantial increase in the permeability coefficients of hydrocortisone propionate (**1h**), hydrocortisone hexanoate (**1j**), and the highly polar sucrose molecule. The permeability of sucrose through delipidized stratum corneum approaches the values for the more lipophilic hydrocortisone esters and all of the values are similar to those reported by Scheuplein and Ross for the permeability coefficients of propanol (0.23 cm/hr) and heptanol (0.24 cm/hr) through delipidized stratum corneum.

Transport through delipidized stratum corneum does not appear to be strictly aqueous boundary layer controlled. Assuming a boundary layer thickness of 300 μm for the diffusion cell utilized (4,32) and an aqueous diffusivity of $5.7 \times 10^{-6}$ cm²/sec for the hydrocortisone esters [estimated from the hydrocortisone data of Stout *et al.* (33) at 25°C by adjusting to 37°C using the Stokes–Einstein equation], a permeability coefficient of 0.68 cm/hr is estimated for aqueous boundary layer-controlled transport, a value somewhat higher than those reported in Table I. It is reasonable to expect that diffusion through delipidized stratum corneum may not be boundary layer controlled but rather may be analogous to diffusion through a microporous membrane (34), which is governed by the effective pore size of the membrane and the tortuosity.

Recent observations of Elias *et al.* (8) in human skin and Smith *et al.* (9) in reaggregated stratum corneum suggest that there is no relationship between barrier function and the number of cell layers or the thickness of stratum corneum, but an inverse relationship exists between lipid content and permeability. Data obtained in this laboratory for the permeability coefficient of hydrocortisone propionate versus stratum corneum lipid content (Fig. 2) do not support an inverse relationship between permeability and lipid content. Other variables such as the lipid composition and the organization of lipids in the stratum corneum, rather than the total lipid content, may be more important.

### Solute Permeability–Uptake Relationships

Although widely applied in skin permeation studies, the assumption implicit in Eq. (1) that a linear relationship exists between permeability coefficients and solute membrane/water partition coefficients has been questioned by Scheuplein *et al.* (6), who found for a group of steroids that the $K_m$ changed by only 15-fold, in comparison to a 100-fold change in permeability constants. Because even the most polar molecules in that series had a significant affinity for stratum corneum, they concluded that "the lesser permeability of the polar steroids does not arise from a limited solubility within the membrane but from their decreased mobility (smaller $D_m$) due to stronger chemical binding." Yotsuyanagi and Higuchi (35), however, proposed that a two-phase model for the stratum corneum with a relatively constant membrane diffusion coefficient for all solutes in the series could also account for the data of Scheuplein *et al.*, but the

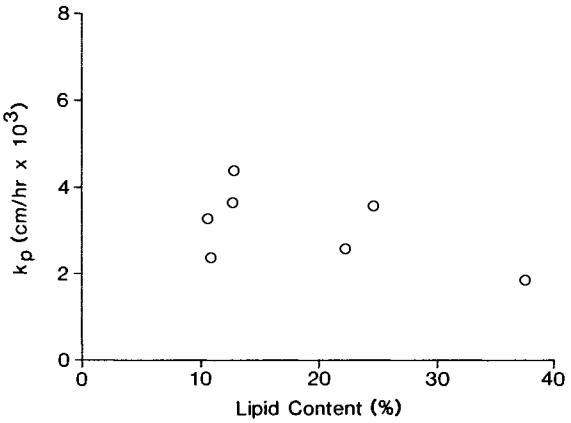



Fig. 2. Permeability coefficients of hydrocortisone propionate versus stratum corneum lipid content (% w/w) at 37°C.

lack of data for the partition coefficients into the two domains precluded a rigorous test of the theory.

Previous studies in this laboratory (14) seem to indicate that, depending on the solute lipophilicity or the structure of the solute, the uptake of hydrocortisone 21-esters into stratum corneum may be governed by the protein or the lipid domains or a combination of the two. Due to differences in the selectivities of the two domains, a change in the mechanism of uptake from protein domain dominated for hydrophilic solutes to lipid domain dominated for lipophilic solutes was observed. Thus, linearity between solute fluxes and partition coefficients in stratum corneum would not be expected.

Table II presents transport data obtained for various esters of hydrocortisone at 37°C through isolated human stratum corneum. The range of permeability coefficients is from $2.6 \times 10^{-5}$ cm/hr for hydrocortisone succinamate (**1a**) to $6.2 \times 10^{-2}$ cm/hr for hydrocortisone octanoate (**1k**)—a change of 2400-fold. Stratum corneum/water partition coefficients for the same compounds vary by 100- to 800-fold, depending on the stratum corneum lipid content (14), indicating that the permeability coefficients are much more sensitive to structural changes than are the partition coefficients. The relationship between the permeability coefficients and the stratum corneum/water partition coefficients is clearly seen in Fig. 3, where the logs of permeability coefficients are plotted against the logs of partition coefficients for the various hydrocortisone esters studied. It is clear from this plot that, contrary to Eq. (1), the relationship between permeabilities and partition coefficients is distinctly nonlinear.

This nonlinear, concave downward relationship is a consequence of the heterogeneous nature of the stratum corneum. A simple model which is consistent with the observations in Fig. 3 assumes that the permeability coefficient, $k_p$, is governed by the contributions of two barriers in series as described by Eq. (2):

$$k_p = \frac{1}{1/k_{p(lip)} + 1/k_{p(polar)}} \tag{2}$$

where

$$k_{p(lip)} = \frac{D_{lip}K_{lip}}{\lambda} \tag{3}$$

**Table II.** Permeability Coefficients ($k_p$) of Various Hydrocortisone 21-Esters Through Human Stratum Corneum at 37°C

| Compound[a] | $k_p$ (cm/hr ± % SE) | $N$ |
|---|---|---|
| **1a** | $2.6 \times 10^{-5}$ (±10%) | 2 |
| **1b** | $6.7 \times 10^{-5}$ (±10%) | 2 |
| **1c** | $2.1 \times 10^{-4}$ (± 5%) | 2 |
| **1d** | $6.3 \times 10^{-4}$ (±14%) | 2 |
| **1e** | $1.8 \times 10^{-3}$ (±43%) | 2 |
| **1f** | $8.9 \times 10^{-4}$ (±46%) | 2 |
| **1g** | $9.1 \times 10^{-4}$ (±31%) | 2 |
| **1h** | $3.4 \times 10^{-3}$ (±11%) | 8 |
| **1i** | $5.4 \times 10^{-3}$ (±18%) | 2 |
| **1j** | $1.8 \times 10^{-2}$ (±30%) | 3 |
| **1k** | $6.2 \times 10^{-2}$ (±12%) | 2 |

[a] See Scheme I.

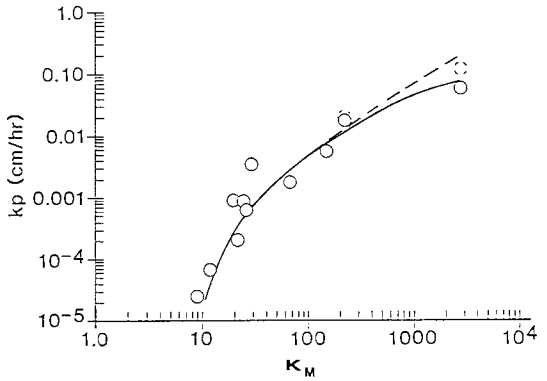



Fig. 3. A log–log plot of the permeability coefficients of hydrocortisone esters versus their stratum corneum/water partition coefficients. The solid line represents the results of nonlinear least-squares regression according to Eqs. (2), (3), (5), and (6).

and $k_{p(polar)} = 0.127$ cm/hr [calculated by averaging the **1h** and **1j** permeability coefficients in delipidized stratum corneum (Table I)]. $k_{p(polar)}$ accounts for the resistance due to aqueous layers in series with the lipid pathway, including boundary layers, and contributes significantly only to the permeability coefficients of the hexanoate and octanoate esters. The $k_{p(lip)}$ values obtained by correcting the $k_p$ data for the contribution from $k_{p(polar)}$ are also plotted in Fig. 3 (dashed lines). The concave downward curvature remains even after correcting for the contribution of aqueous diffusion, indicating that the shape of the $k_p$ curve is not due to a change in the rate-determining step from lipid bilayer diffusion to diffusion through aqueous layers, although such a change does occur at higher lipophilicities.

The important diffusion constant and partition coefficient in this model, $D_{lip}$ and $K_{lip}$, reflect properties of the rate-limiting microenvironment of the transport barrier. We assume, for the purpose of illustrating the concept, that the selectivity of this rate-limiting domain can be related to a bulk solvent partitioning system such as the octanol/water system through a linear free energy relationship (36,37) described by Equation (4).

$$\log(K_{lip}) = \beta\log(K_{o/w}) + c \tag{4}$$

or

$$K_{lip} = \alpha K_{o/w}^{\beta} \tag{5}$$

where $K_{o/w}$ is the octanol/water partition coefficient of the solute of interest, and $\beta$ and $c$ ($=\log\alpha$) are constants. $\beta$ reflects the relative selectivity of the barrier microenvironment to solute structure; i.e., the larger the value of $\beta$, the greater the differences in permeability as the solute lipophilicity, measured by the octanol/water partition coefficient, is varied. A $\beta$ equal to one would indicate that the barrier selectivity closely resembles that of octanol. As described, $K_{lip}$ need not reflect the equilibrium partition coefficient into the lipid domain of the stratum corneum, since the properties of the barrier microenvironment may not parallel those of the entire lipid domain.

Stratum corneum/water partition coefficients, $K_m$, reflect both lipid and protein domains (14). We have previously described the selectivities of these domains in terms

of linear free energy relationships using octanol/water partition coefficients, which form a convenient scale of lipophilicity. Combining the contributions of both domains, we obtain Eq. (6):

$$K_m = W_{f(prot)} * \gamma K_{o/w}^{\delta} + W_{f(lip)} * \epsilon K_{o/w}^{\zeta} \quad (6)$$

where the first and second terms represent the contributions of the protein and lipid domains, respectively, to solute uptake into stratum corneum. $W_{f(prot)}$ and $W_{f(lip)}$ are the weight fractions of protein and lipid in the stratum corneum, which average 0.85 and 0.15, respectively. The values of $\gamma$ (=7.4), $\delta$ (=0.24), $\epsilon$ (=0.15), and $\zeta$ (=0.91) were determined in a previous paper (14).

The solid line in Fig. 3 represents the results of nonlinear least-squares regression (MINSQ, MicroMath Inc., Salt Lake City, Utah) for $k_p$ versus $K_{o/w}$ according to Eqs. (2), (3), and (5) and $K_m$ versus $K_{o/w}$ according to Eq. (6). $k_p$ is therefore an implicit function of $K_m$. The model described appears to account adequately for the nonlinearity of the curve. The values obtained for the fitted parameters in the model, $\alpha D_{lip}/\lambda$ (all three of these terms are unknown, but since their values cannot be ascertained, they are combined as a single unknown) and $\beta$ [Eqs. (3) and (5)], are $1.13 \times 10^{-6}$ cm/hr and 0.85, respectively. Note that the rate-limiting microenvironment sensitivity parameter, $\beta$, is close to one. Thus, the rate-limiting microenvironment in the lipid bilayer region of the stratum corneum appears to be similar in polarity to octanol.

Qualitatively, the nonlinearity of Fig. 3 results primarily from the fact that the permeability coefficients are much more sensitive to structure than the composite partition coefficients for those relatively hydrophilic solutes for which equilibrium uptake is protein domain dominated (left side of Fig. 3). The slope decreases (upper right region) for lipophilic solutes that partition preferentially into the lipid domain, which is more sensitive to solute lipophilicity than the protein domain. The transition from partitioning behavior dominated by the protein domain to partitioning behavior dominated by the lipid domain comes at about a partition coefficient of 50. Clearly, these results support a two-phase model of the stratum corneum. Marked decreases in diffusion coefficients with the inclusion of additional polar groups, as suggested by Scheuplein *et al.* (6), are not required to account for the observed permeabilities. [While molecular size would be expected to alter diffusivity significantly (38), the change in molecular weight within the series of hydrocortisone esters examined was less than 20%. Consequently, molecular size effects were ignored in this treatment.]

### Functional-Group Contributions to Permeability and Uptake

Functional-group contributions to the standard free energy of transfer from water to heptane, octanol, and the stratum corneum protein and lipid domains were obtained from equilibrium partition coefficient measurements and Eq. (7):

$$\Delta(\Delta G^{\circ})_{-X} = -2.303RT\log(K_{RX}/K_{RH}) \quad (7)$$

Heptane/water, octanol/water, stratum corneum protein domain/water, and lipid domain/water partition coefficients for various hydrocortisone esters are reported in Table III.

Functional-group contributions to the standard free energy of transfer of solute from water to the rate-limiting microenvironment of the stratum corneum were obtained from ratios of $k_p$ values for substituted and unsubstituted esters, in a manner similar to that depicted in Eq. (7). This calculation assumes that diffusion coefficients were not changed on substitution. In an attempt to minimize variability in the group contribution values due to variation in barrier properties of stratum corneum derived from different sources, the permeability coefficient of hydrocortisone propionate was determined in every experiment along with other solutes of interest. Permeability coefficients were then normalized to

**Table III.** Heptane/Water, Octanol/Water, Stratum Corneum Protein/Water, and Stratum Corneum Lipid Domain/Water Partition Coefficients of Various Hydrocortisone 21-Esters (Scheme I) at 37°C

| | Partition coefficient[a] | | | |
|---|---|---|---|---|
| | | | Stratum corneum | |
| Compound | Heptane water | Octanol water | Protein domain water | Lipid domain water[b] |
| **1a** | — | 27 ± 1% (2) | 13 ± 15% (14) | 14 ± 7% |
| **1b** | $1.2 \times 10^{-4}$ ± 12% (6) | 108 (1) | 17 ± 12 (14) | — |
| 1c | $4.7 \times 10^{-3}$ ± 4 (2) | 380 ± 1 (2) | 22 ± 9 (14) | — |
| **1d** | — | 130 ± 2 (2) | 39 ± 10 (2) | 14 ± 14 |
| 1e | $8.2 \times 10^{-5}$ ± 32 (2) | 1800 (1) | 71 ± 3 (2) | — |
| **1f** | — | 200 ± 0 (2) | 28 ± 4 (4) | — |
| **1g** | $0.90 \times 10^{-4}$ ± 13 (6) | 610 ± 1 (2) | 23 ± 0 (4) | 38 ± 5 |
| **1h** | $3.2 \times 10^{-2}$ ± 4 (2) | 990 ± 7 (2) | 22 ± 9 (25) | 69 ± 5 |
| **1i** | $7.0 \times 10^{-2}$ ± 11 (2) | 5000 ± 6 (4) | 66 ± 21 (3) | 530 ± 20 |
| **1j** | 1.32 ± 2 (2) | 30000 ± 11 (2) | 62 ± 24 (6) | 1600 ± 26 |
| **1k** | 20 ± 11 (2) | $3.1 \times 10^{5}$[c] | 550 ± 11 (7) | 16000 ± 3 |

[a] Mean ± % SE of the number of determinations indicated in parentheses.

[b] Values for **1a**, **1d**, and **1g** were measured directly using extracted stratum corneum lipids ($N = 2$) (see Ref. 40). Other values are averages (±% SE) of the partition coefficients measured directly in extracted stratum corneum lipids and those determined from the difference in partition coefficients between untreated and delipidized stratum corneum (see Refs. 14 and 40).

[c] Estimated value (Ref. 14).

Table IV. Thermodynamic Group Contributions (cal/mol) to the Free Energy of Transfer of Various Functional Groups from Water to Hydrocarbon, Octanol, Stratum Corneum, or the Stratum Corneum Barrier Microenvironment at 37°C

| Functional group | Heptane[a] | Octanol[a] | Stratum corneum | | |
|---|---|---|---|---|---|
| | | | Protein domain[a] | Lipid domain[a] | Transport barrier[b] |
| $-CH_2-$ | −800 (−850)[c] | −720 (−710)[d] | −210[e] | −680 | −440 |
| $-CONH_2$ | 6600 (6400)[f] | 2700 (3100)[d] | 410 | 1000 | 2700 |
| $-CON(CH_3)_2$ | 3500 | 1400 | 160 | — | 2600 |
| $-COOCH_3$ | 1500 | 850 (1200)[d] | ~0 | 670 | 1400 |
| $-COOH$ | 6000 | 1600 (1700)[d] | −220 | 1000 | 1500 |
| $-OH$ | 5900 (5600)[g] | 2400 (2500)[d] | 610 | 2300 | 2400 |

[a] Calculated according to Eq. (7).
[b] $\Delta(\Delta G^{\neq})_{-X} = -2.303RT\log(k_p[RX]/k_p[RH])$.
[c] 25°C data from Ref. 39.
[d] 25°C data from Ref. 43.
[e] The octanoate ester (**1k**) was not included in this calculation since a large contribution to the partition coefficient from a small percentage of residual unextracted lipids was suspected.
[f] 25°C data from Ref. 41.
[g] 25°C data from Ref. 42.

hydrocortisone propionate. The group contributions so obtained are reported in Table IV.

Selectivity in partitioning refers to the relative ability of two solvents to discriminate between solutes (36,37). Higher selectivity is manifested in a greater disparity between functional-group contributions. As is well known (39), selectivity in bulk solvent/water partitioning decreases with increasing polarity of the organic phase. This is evident in a comparison of octanol/water partition coefficients with those in heptane/water (Table III). It is of interest to compare the stratum corneum values with group contributions in bulk solvent systems, therefore, to ascertain the functional polarity of the stratum corneum. In equilibrium uptake experiments, the stratum corneum protein domain appears to be significantly more polar than octanol. Although group contributions for uptake into the stratum corneum lipid domain were obtained from only a limited number of comparisons, the data confirm that the lipid domain is significantly less polar than the protein domain.

With respect to the values of the group contributions obtained from transport studies, the apparent polarity of the stratum corneum barrier microenvironment varies significantly depending on the nature of the functional group used as a probe. Thus, $-CH_2-$ contributions to transport are substantially smaller than the corresponding values in octanol/water partitioning, while functional groups which can both donate and accept hydrogen bonds ($-CONH_2$, $-COOH$, $-OH$) exhibit similar group contributions in transport experiments and octanol/water partitioning. Hydrogen acceptor functional-group [$-CON(CH_3)_2$ and $-COOCH_3$] contributions more closely resemble those found in heptane/water partitioning experiments. Most interesting, perhaps is the finding that the selectivity of the stratum corneum barrier toward polar hydrogen-bonding groups is similar to that of the relatively polar, hydrogen-bonding solvent, octanol, and is significantly less than that of heptane. This suggests that hydrogen bonds are not completely broken in the transition state for passive diffusion via the lipid pathway.

## ACKNOWLEDGMENTS

This work was supported by a grant from Riker Laboratories, Inc./3M, St. Paul, Minnesota. Human stratum corneum samples were obtained through Drs. Gerald Krueger and Lynn Pershing, University of Utah. We also gratefully acknowledge the assistance of Scott Smith in generating heptane/water partition coefficients.

## REFERENCES

1. R. J. Scheuplein. *J. Invest. Dermatol.* 48:79–88 (1967).
2. R. J. Scheuplein and I. J. Blank. *Physiol. Rev.* 51:702–747 (1971).
3. S. K. Chandrashekaran and J. E. Shaw. *Curr. Probl. Dermatol.* 7:172–186 (1978).
4. G. L. Flynn, H. Durrheim, and W. I. Higuchi. *J. Pharm. Sci.* 70:52–56 (1981).
5. G. L. Flynn. In R. Bronaugh and H. Maibach (eds.), *Mechanism of Percutaneous Absorption from Physicochemical Evidence*, Marcel Dekker, New York, 1985, pp. 17–42.
6. R. J. Scheuplein, I. H. Blank, G. D. Brauner, and D. J. MacFarlane. *J. Invest. Dermatol.* 52:63–70 (1969).
7. A. J. Michaels, S. K. Chandrasekaran, and J. E. Shaw. *AIChE J.* 21:985–996 (1975).
8. P. M. Elias, E. R. Cooper, A. Korc, and B. E. Brown. *J. Invest. Dermatol.* 76:297–301 (1981).
9. W. P. Smith, M. S. Christenson, S. Nacht, and E. H. Gans. *J. Invest. Dermatol.* 78:7–11 (1982).
10. P. M. Elias. *Arch. Dermatol. Res.* 270:95–117 (1981).
11. P. M. Elias and D. S. Friend. *J. Cell Biol.* 65:180–191 (1975).
12. P. M. Elias, B. E. Brown, P. Fritsch, J. Goerke, G. M. Gray, and R. J. White. *J. Invest. Dermatol.* 73:339–348 (1979).
13. P. M. Elias, J. Goerke, and D. S. Friend. *J. Invest. Dermatol.* 69:535–546 (1977).
14. P. V. Raykar, M. Fung, and B. D. Anderson. *Pharm. Res.* 5:140–150 (1988).
15. A. M. Kligman and E. Christophers. *Arch. Dermatol.* 88:702–705 (1963).
16. L. Juhlin and W. B. Shelly. *Acta Derm. Venereol.* 57:289–96 (1977).
17. E. G. Bligh and W. J. Dyer. *Can. J. Biochem. Physiol.* 37:911–917 (1959).
18. R. Scheuplein and L. Ross. *J. Soc. Cosmet. Chem.* 21:853–873 (1970).

19. A. Leo, C. Hansch, and D. Elkins. *Chem. Rev.* 71:525–554 (1971).
20. H. Durrheim, G. L. Flynn, W. I. Higuchi, and C. R. Behl. *J. Pharm. Sci.* 69:781–786 (1980).
21. T. A. Hagen and G. L. Flynn. *J. Membr. Sci.* 30:47–65 (1987).
22. D. D. Perrin. *Aust. J. Chem.* 16:572–578 (1963).
23. C. Ackerman, G. L. Flynn, and W. M. Smith. *Int. J. Pharm.* 36:67–71 (1987).
24. M. M. Saket, K. C. James, and I. W. Kellaway. *Int. J. Pharm.* 27:287–298 (1985).
25. B. D. Anderson. In S. H. Yalkowsky, A. A. Sinkula, and S. C. Valvani (eds.), *Physical Chemical Properties of Drugs,* Marcel Dekker, New York, 1980, Chap. 7.
26. W. L. Hubbell and H. M. McConnell. *J. Am. Chem. Soc.* 93:314 (1971).
27. J. Seelig and A. Seelig. *Q. Rev. Biophys.* 13:19–61 (1980).
28. J. A. Marqusee and K. A. Dill. *J. Chem. Phys.* 85:434–444 (1986).
29. L. DeYoung and K. A. Dill (submitted for publication).
30. W. M. Smith. *An Inquiry into the Mechanism of Percutaneous Absorption of Hydrocortisone and Its 21-n-Alkyl Esters,* Ph.D. thesis, University of Michigan, Ann Arbor, 1982.
31. W. J. Lambert. Ph.D. thesis, University of Utah, Salt Lake City, 1988.
32. J. L. Fox, G. L. Flynn, T. Hagen, W. I. Higuchi, N. F. H. Ho, and H. Durrheim. *Abstracts, APhA Academy of Pharmaceutical Sciences, Vol. 8 (2),* Nov. 1978, p. 100.
33. P. J. M. Stout, N. Khoury, J. Mauger, and S. Howard. *J. Pharm. Sci.* 75:65–67 (1986).
34. K. A. Johnson, G. B. Westermann-Clark, and D. O. Shah. *J. Pharm. Sci.* 76:277–285 (1987).
35. T. Yotsuyanagi and W. I. Higuchi. *J. Pharm. Pharmacol.* 24:934–941 (1972).
36. Y. Katz, M. E. Hoffman, and R. Blumenthal. *J. Theor. Biol.* 105:493–510 (1983).
37. J. M. Diamond and Y. Katz. *J. Membr. Biol.* 17:121–154 (1974).
38. W. R. Lieb and W. D. Stein. In W. D. Stein (ed.), *Transport and Diffusion Across Cell Membranes,* Academic Press, New York, 1986, Chap. 2.
39. S. S. Davis, T. Higuchi, and J. H. Rytting. In H. S. Bean, A. H. Beckett, and J. E. Carless (eds.), *Advances in Pharmaceutical Sciences, Vol. 4,* Academic Press, New York, 1974.
40. P. Raykar. *Stratum Corneum Domain Effects on Solute Uptake and Transport,* Ph.D. thesis, University of Utah, Salt Lake City, 1988.
41. B. D. Anderson and R. A. Conradi. *J. Pharm. Sci.* 69:424–430 (1980).
42. J. H. Rytting, L. P. Huston, and T. Higuchi. *J. Pharm. Sci.* 67:615–618 (1978).
43. J. Iwasa, T. Fujita, and C. Hansch. *J. Med. Chem.* 8:150 (1965).