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Short chain alkanols as transport enhancers for lipophilic and polar/ionic permeants in hairless mouse skin: Mechanism(s) of action

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Summary

The influences of short chain *n*-alkanols (from C1 to C5) and isopropanol on the transport of lipophilic (β-estradiol and hydrocortisone) and polar/ionic (tetraethylammonium ion) permeants across hairless mouse skin have been investigated. Permeability studies employing a two-chamber diffusion cell were carried out over wide ranges of alkanol (in saline) concentrations with an aim toward quantifying the reversible enhancement effects of the added alkanol upon the lipoidal pathway of the stratum corneum. An enhancement factor, E (for the lipoidal pathway of the stratum corneum), was calculated from permeability coefficient and solubility data, and the E values for β-estradiol and for hydrocortisone were found to be nearly always the same in all instances. A pattern of increasing E values with increasing alkanol chain length up to C5 with these two permeants was found. A nearly semi-logarithmic linear relationship was also obtained between the enhancement potency and the carbon number of the *n*-alkanols; there was about 4-fold increase in the enhancement potency per *n*-alkanol methylene group. Pretreatment studies showed that the *n*-alkanol effects at low concentrations were reversible as far as the lipoidal pathway of the stratum corneum was concerned. These results demonstrate the general usefulness of this approach for evaluating the action of enhancers on the barrier function of the stratum corneum. It is suggested that the short chain alkanols may work at low concentrations as effective 'fluidizing' agents at some locus in the stratum corneum lipid bilayer at or near the polar head plane, but not in the deep bilayer hydrocarbon interiors.

Introduction

Recently, the influence of ethanol in aqueous solutions upon the transport behavior of several lipophilic and hydrophilic/ionic permeants in hairless mouse skin was investigated (Ghanem et al., 1986, 1987a,b; Higuchi et al., 1987). A theoretical model was developed and the transport enhancement factor, E, for the lipoidal pathway of the stratum corneum was calculated using experimental permeation data obtained at low ethanol concentrations ($\leq 25\%$) with estrone, β-

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estradiol (E2β) and hydrocortisone (HC) as probe permeants. The experimental permeability coefficient for full thickness hairless mouse skin was corrected for the dermis/epidermis permeability coefficient and stratum corneum pore pathway permeability coefficient. Solubility data were used to account for chemical potential changes with solvent composition, assuming Henry's law which was later experimentally validated (Kim et al., 1989; Ghanem et al., 1992). The E values for estrone, E2β, and HC were found to be of the same magnitude (of around 7–9 at 25% and of the order of 100 at 50% ethanol). In addition to these results, it was found that, at very high ethanol concentrations, pore pathway dominated permeation of all solutes across the stratum corneum irrespective of their polarity. At low ethanol levels ($\leq 25\%$), ethanol had little or no effect on the pore pathway; this was inferred from the only slight changes found in the permeability coefficients of polar-ionic compounds [tetraethylammonium bromide (TEAB), mannitol, vidarabine and estrone ammonium sulfate] in going from saline to 25% ethanol.

The promising results with ethanol as a transport enhancer led to our desire to examine the effects of other *n*-alkanol members of the homologous series on the transport of the same permeants through hairless mouse skin. Recently, *n*-alkanols as possible transport enhancers have been investigated in vitro for their potency and mechanism (Friend et al., 1988; Kai et al., 1990; Knutson et al., 1990). The alkanols used in the present study besides ethanol were: methanol, *n*-propanol, *n*-butanol, *n*-pentanol in addition to a branched alkanol member, isopropanol, which is a widely used vehicle in pharmaceutical formulations.

The purpose of the present investigation was two-fold. Firstly, it was to demonstrate the general usefulness of our approach with other enhancers, especially to generalize the procedure for calculating E values from experimental permeation data. Secondly, it was to investigate the mechanism of skin permeation enhancement of the lipoidal pathway induced by the short chain *n*-alkanols.

Considerations in the Determination of the Enhancement Factor, E

For the model shown in Fig. 1, the total permeability coefficient, P_T, for a solute is defined as:

$$1/P_T = 1/P_{SC} + 1/P_{D/E} \quad (1)$$

where P_{SC} and $P_{D/E}$ represent the permeability coefficients of the stratum corneum and the dermis and epidermis combination, respectively. The permeability coefficient of the stratum corneum, P_{SC}, is assumed to be the summation of a lipoidal pathway and a pore pathway as follows:

$$P_{SC} = P_L + P_P \quad (2)$$

where P_L and P_P denote the respective permeability coefficients of the lipoidal pathway and the pore pathway which may be determined experimentally using highly polar compounds such as TEAB, mannitol, estrone ammonium sulfate, or vidarabine.

If the lipoidal pathway of the stratum corneum is treated as a homogeneous barrier, the permeability coefficient, P_L, may be defined as:

$$P_L = KD/h \quad (3)$$

where K represents the stratum corneum lipid-to-solvent partition coefficient, D is the diffusion coefficient in the stratum corneum lipid, and h denotes the effective thickness of the stratum corneum.

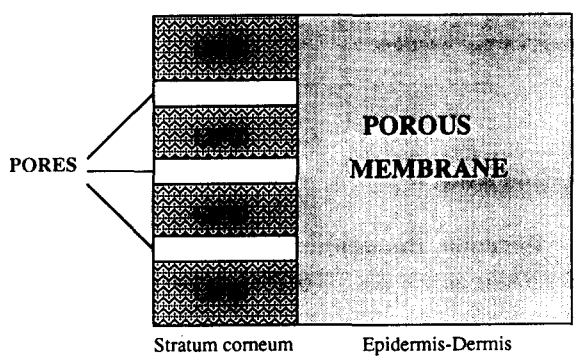

Fig. 1. Theoretical skin model for data analysis.

Consider now the situation involving a two-chamber diffusion cell experiment where the solvent compositions in the donor and the receiver chambers are the same. Let $P_{L,O}$ be the permeability coefficient of the solute in the saline case and $P_{L,X}$ be the permeability coefficient of the solute when the solvent is aqueous alkanol at a concentration, X. We may then write the following ratio:

$$(P_{L,X}/P_{L,O}) = (K_X/K_O)(D_X/D_O) \quad (4)$$

where K_X represents the partition coefficient when the solvent is aqueous alkanol at concentration, X, K_O is the partition coefficient when the solvent is saline, D_X denotes the diffusion coefficient in the stratum corneum lipid when the solvent is aqueous alkanol at concentration, X, and D_O is the diffusion coefficient in the stratum corneum lipid when the solvent is saline.

We may further write:

$$K_X/K_O = (K_X/K_O)_S F \quad (5)$$

Here, $(K_X/K_O)_S$ is the hypothetical partition coefficient ratio which corresponds to the case where the solute partitioning tendencies in the stratum corneum lipid phase (at constant solute chemical potential) are the same for the saline and for the alkanol/saline cases. In general, however, the solute partitioning tendencies in the lipid phase may not be the same when saline is the solvent and when an alkanol/saline mixture is the solvent. F is the factor which corrects for this difference in the solute partitioning tendencies, and it may therefore have values other than unity.

By combining Eqns 4 and 5, we have:

$$F(D_X/D_O) = (P_{L,X}/P_{L,O})(K_O/K_X)_S \quad (6)$$

The right-hand side of Eqn 6 may be determined experimentally. $P_{L,X}$ and $P_{L,O}$ are obtained from the two-chamber diffusion cell experiments and by using Eqns 1 and 2. The ratio, $(K_O/K_X)_S$, may be conveniently obtained from solubility data according to:

$$(K_O/K_X)_S = S_X/S_O \quad (7)$$

where S_X and S_O are the solute solubilities in alkanol/saline and saline, respectively.

Eqn 7 is expected to be valid if Henry's law is obeyed for the solute up to saturation concentration levels.

We may now define the left-hand side of Eqn 6 as the enhancement factor, E,

$$E = F(D_X/D_O) \quad (8)$$

The significance of E is that it accounts for both the thermodynamic factor, F, and the kinetic factor, (D_X/D_O). While E may be determined from experimental permeability coefficients and solubility data (i.e., by using Eqns 6 and 7), other kinds of studies are clearly required for factoring out E in terms of F and (D_X/D_O).

Materials and Methods

Materials

β-[^{3}H]Estradiol, [^{3}H]hydrocortisone, and [^{14}C]TEAB (New England Nuclear, Boston, MA) were checked for purity by the method specified by the supplier and were judged to be more than 98% pure prior to the experiments. Unlabeled E2β from Sigma Chemical Co. (St. Louis, MO) and unlabeled HC from Fluka AG (Buchs, Switzerland) were used. Methanol (Baker Chemical Co., Phillipsburg, NJ), ethanol (US Industrial Chemical Co., Tuscula, IL), *n*-propanol, *n*-butanol, *n*-pentanol (Aldrich Chemical Co., Milwaukee, WI), isopropanol (Sigma Chemical Co., St. Louis, MO), and normal saline (McGaw, Irvine, CA) were used as received. The purity of all solvents was checked using GC and was found to be more than 98%.

Male hairless mice strain SKH-HR1, 8–12 weeks old, were obtained from Temple University, Pittsburgh, PA.

Methods

Permeability experiments

Permeability experiments were carried out as previously described (Ghanem et al., 1987b) using

a two-chamber diffusion cell of 2 ml volume of each compartment and an effective diffusional area of 0.67 cm^2. Briefly, for experiments with full thickness skin, freshly separated, hairless mouse skin obtained from the abdomen region and freed from adhering fat and other visceral debris was sandwiched between the half cells, and 2 ml of the alkanol/saline solvent mixture was pipetted into both the donor and receiver chambers and allowed to equilibrate at 37°C. An appropriate amount of the radiolabeled solute (at a radioactive tracer level, 10 000 dpm/10 μl which corresponds to concentrations far below solubility saturation: 0.1% saturation solubility for E2β and 0.001% saturation solubility for HC in saline) was then added to the donor chamber. Samples were withdrawn from both donor and receiver chambers at predetermined time intervals and mixed with 10 ml scintillation cocktail (Opti-Fluor, Packard Instrument Co., Downers Grove, IL) and analyzed using a Beckman Liquid Scintillation Counter (Model LS-7500). Usually, 3- or 10-μl aliquots were taken from the donor chamber and 100- or 500-μl aliquots from the receiver chamber. The same volume of the fresh vehicle was added back to the receiver chamber to maintain constant volume. The total permeability coefficient, P_T, was calculated at steady state under sink conditions. Experiments were run long enough so that the steady-state portions were typically around 3–5 times longer than the lag times.

During the course of this investigation, two problems were encountered with regard to the calculation of P_T from the experimental transport data. Both of these pertained only when E2β was the permeant: (a) the necessity to correct for 'dermis retention' of the permeant under the quasi-steady-state conditions of the transport experiments and (b) the need to consider the possible error in calculating P_T for E2β when metabolism is neglected. Both of these problems have now been investigated. When appropriate, corrections for dermis retention have been applied to the present E2β data. The possible effects of E2β metabolism in the calculation of P_T for E2β, however, have not been taken into account; a correction factor of around 15% (which is within the experimental error of the permeability coefficient) was previously estimated for the saline case (Liu et al., 1990).

The permeability experiments with stripped skin were carried out in the same manner, but after stripping the abdominal skin 30 times using a 3M Scotch tape (St. Paul, MN).

Solubility experiments

Excess amounts of non-radiolabeled E2β or HC were shaken for 72 h at 37°C in 1.5 ml polypropylene micro-centrifuge tubes with different alkanol/saline solvent mixtures in a thermostatically controlled water bath (Model YB-521, American Scientific) (Ghanem et al., 1987b). The tubes were centrifuged for 5 min at 2000 rpm (Fischer Micro Centrifuge, Model 235A). The clear supernatant solution was analyzed for drug concentration using HPLC. The HPLC system consisted of a pump (Beckman Institute, San Ramon, CA) with a flow rate of 3 ml/min, UV detector (ISCO, Lincoln, NE) at 280 nm for E2β and 250 nm for HC, a column (Resolvex C18, Fisher Scientific, Pittsburgh, PA) and a 10 μl injector (C.W., Valco Institute, San Antonio, TX). Acetonitrile : water (35 : 65, Baker Chemical Co., Phillipsburgh, NJ) saturated with ether was used as the mobile phase.

Test for reversibility

The diffusion cell was assembled with full thickness hairless mouse skin, as described before, for a typical permeation experiment. However, in this protocol, the skin was first exposed to 2 ml of the alkanol/saline solution present in both chambers for 4 h at 37°C with mild stirring (150 rpm). The solution was then removed from both chambers and the diffusion cell was rinsed thoroughly several times with saline. The permeability experiment was then carried out with saline in both chambers using E2β (highly lipophilic solute), HC (moderately lipophilic solute), and TEAB (ionic solute). The permeability coefficients obtained in saline after treatment with aqueous alkanols were compared with those determined in saline without pretreatment.

Validation of Henry's law

According to Eqn 7, the ratio of the permeant solubility in the alkanol/saline solvent to that in saline is used to estimate the partition coefficient ratio, $(K_O/K_X)_S$. For Eqn 7 to be generally valid (i.e., for any permeant concentration up to saturation), Henry's law must be obeyed for the permeant up to solubility saturation both in the alkanol/saline solvent and in saline.

To examine the validity of Henry's law for E2β and HC solutions, permeability experiments were conducted using E2β and HC as the permeants at tracer levels (radiolabeled E2β or radiolabeled HC) and at saturation (presaturated with non-radiolabeled E2β plus radiolabeled E2β or presaturated with non-radiolabeled HC plus radiolabeled HC). To eliminate the factor of skin-to-skin variation, one piece of skin was used for the tracer level experiment and another piece of the same skin preparation was used for the saturation level experiment. The permeability experiments were carried out as described above.

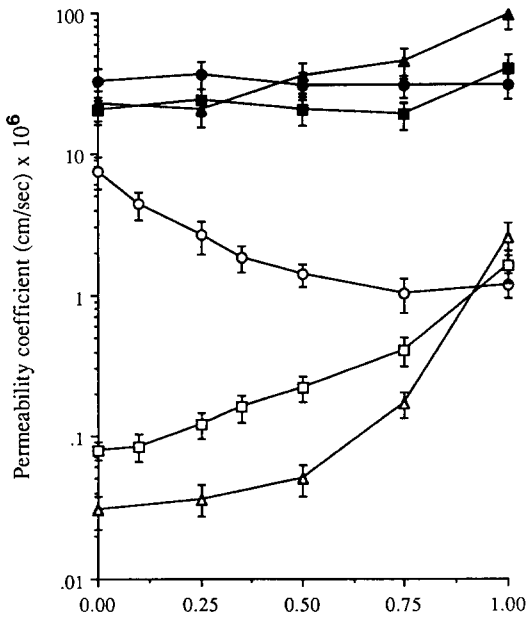

Fig. 2. Permeability coefficients of whole skin (open symbols) and stripped skin (closed symbols) as a function of volume fraction of methanol (MeOH)/saline for β-estradiol (circles), hydrocortisone (squares) and TEAB (triangles), respectively. Each data point represents the mean and standard deviation of four determinations.

Results and Discussion

Influence of the alkanol concentration upon the permeability coefficients

The permeability coefficients for full-thickness skin (P_T) and for stripped skin ($P_{D/E}$) obtained with E2β, HC, and TEAB as the permeants are presented in Figs 2–7 for all of the alkanol/saline systems. The P_T data are indicated by the open symbols and the $P_{D/E}$ data are represented by the closed symbols.

The methanol/saline (Fig. 2) and the ethanol/saline (Fig. 3) results are seen to be similar in many respects. Both sets of data may be interpreted as follows. First, with zero alkanol present (i.e., in saline alone), the very low P_T value for TEAB is believed to be a measure of P_P as pointed out previously (Ghanem et al., 1987b, 1992). The P_T values for E2β and for HC in saline alone are believed to involve mainly the lipoidal pathway of the stratum corneum, and the approx. 100-fold difference between the P_T values for the two permeants reflects the large difference in their lipophilicities. Secondly, at low methanol or ethanol levels (i.e., ≤ 0.25 volume fraction), there is little or no increase in the P_T value of TEAB over that in saline; this is interpreted as being due to small or only modest changes in the pore pathway at low methanol or ethanol concentrations (this assumes that the 'pore' diffusivity of the TEA^+ in saline and in 25% ethanol would be the same). For both systems, however, at higher alkanol concentrations (0.5–0.75), the P_T values for TEAB are appreciably greater than in saline, and one may conclude from this that, at high ethanol or methanol concentrations, there occur significant amounts of new effective pore formation in the stratum corneum. Finally, in 100% ethanol or in 100% methanol, the pore pathway dominates the permeation of full thickness hairless mouse skin and the P_T values become essentially the same for all three permeants, i.e., $P_T \approx 1\text{–}2 \times 10^{-6}$ cm/s in 100% methanol and $P_T \approx 2\text{–}5 \times 10^{-6}$ cm/s in

100% ethanol. It should be noted that these limiting P_T values are yet significantly smaller than the corresponding $P_{D/E}$ values; the stratum corneum is still the rate-limiting component for hairless mouse skin in 100% methanol and in 100% ethanol. It is likely that the large extent of new pore formation in the stratum corneum at high ethanol or methanol levels is a result of some reorganization of the lipid regions, lipid extraction, protein conformational changes, or some combination of these (Kai et al., 1990).

The stripped skin permeability coefficient ($P_{D/E}$) data in Figs 2 and 3 support the previously stated view (Ghanem et al., 1987b, 1992) that the epidermis/dermis behaves as an unchanging porous membrane. The inability of the epidermis/dermis to discriminate between lipophilic (E2β) and ionic (TEAB) permeants over the entire alkanol concentration range is consistent with the idea that the epidermis/dermis is sponge-like and capable of accepting whatever solvent compositions are present in the chambers of the diffusion cell. Also, the nearly constant $P_{D/E}$ values for all alkanol concentrations are consistent with a porous membrane model for the epidermis/dermis (caveat: this again assumes that the permeant diffusivity is relatively constant). While the epidermis/dermis is sponge-like (i.e., a porous membrane) at all methanol or ethanol concentrations, full thickness skin exhibits this character only at 100% methanol or 100% ethanol.

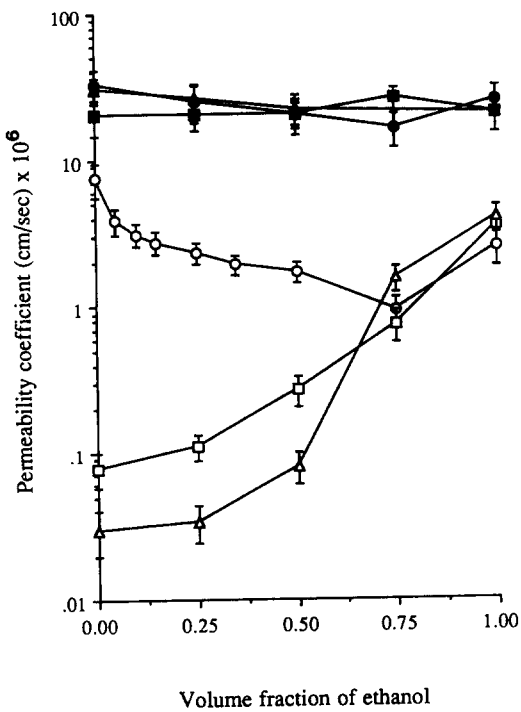

Fig. 3. Permeability coefficients of whole skin (open symbols) and stripped skin (closed symbols) as a function of volume fraction of ethanol (EtOH)/saline for β-estradiol (circles), hydrocortisone (squares) and TEAB (triangles), respectively. Each data point represents the mean and standard deviation of four determinations.

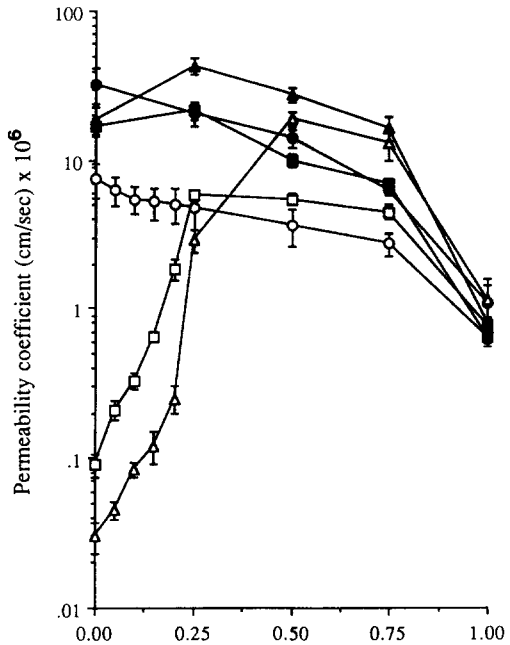

Fig. 4. Permeability coefficients of whole skin (open symbols) and stripped skin (closed symbols) as a function of volume fraction of *n*-propanol (n-POH)/saline for β-estradiol (circles), hydrocortisone (squares) and TEAB (triangles), respectively. Each data point represents the mean and standard deviation of four determinations.

The *n*-propanol/saline data (Fig. 4) and the isopropanol/saline results (Fig. 5) appear to be similar but they differ from the methanol/saline and the ethanol/saline data. For both the *n*-propanol/saline and isopropanol/saline systems, the

P_T values for TEAB increased over that in saline even at relatively low alkanol concentrations, suggesting that the pore pathway is affected at relatively low levels of *n*-propanol and isopropanol, in contrast to the behavior of TEAB in methanol/saline and ethanol/saline systems when the alkanol concentration was varied. The more unusual aspect of the results with the *n*-propanol and isopropanol systems is the significantly large decrease in $P_{D/E}$ at the high alkanol concentrations. At high *n*-propanol or isopropanol levels (0.75 to 1.0) the $P_{D/E}$ values are considerably smaller than those in saline and the simple porous membrane model for the epidermis/dermis seems to become untenable. Moreover, the P_T and $P_{D/E}$ values for TEAB at these high alkanol concentrations are nearly the same (especially for *n*-propanol). These data therefore

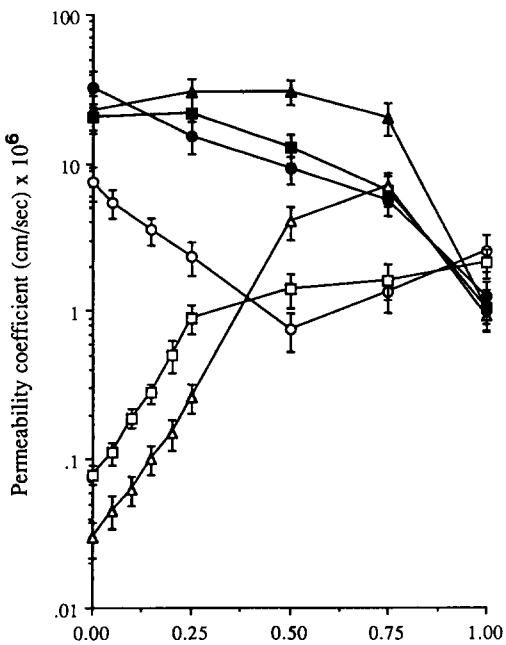

Fig. 5. Permeability coefficients of whole skin (open symbols) and stripped skin (closed symbols) as a function of volume fraction of isopropanol (iso-POH)/saline for β-estradiol (circles), hydrocortisone (squares) and TEAB (triangles), respectively. Each data point represents the mean and standard deviation of four determinations.

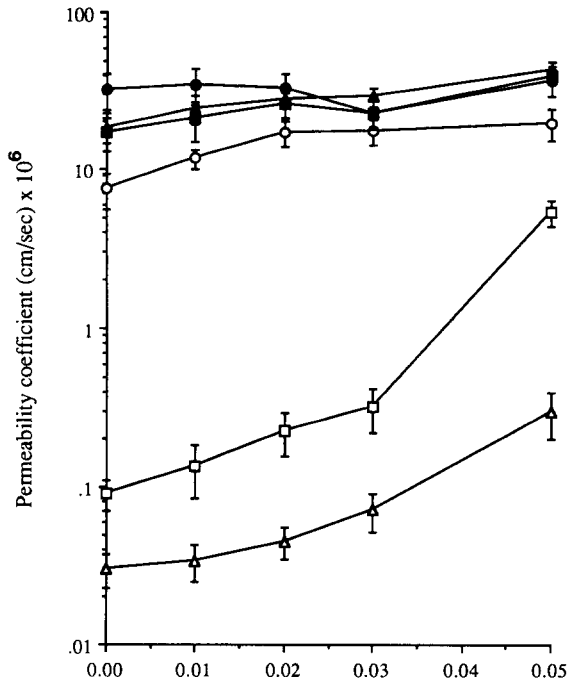

Fig. 6. Permeability coefficients of whole skin (open symbols) and stripped skin (closed symbols) as a function of volume fraction of *n*-butanol (n-BtOH)/saline for β-estradiol (circles), hydrocortisone (squares) and TEAB (triangles), respectively. Each data point represents the mean and standard deviation of four determinations.

suggest that, in the *n*-propanol and isopropanol systems, the epidermis/dermis becomes the transport rate-limiting component of hairless mouse skin. More independent studies are needed for a mechanistic understanding of this unexpected behavior.

The P_T and $P_{D/E}$ results for the *n*-butanol and *n*-pentanol systems are presented in Figs 6 and 7. The concentration ranges investigated were limited in these systems by the solubilities in saline (~ 0.083 and ~ 0.032 for *n*-butanol and *n*-pentanol, respectively). The general behavior of P_T and that of $P_{D/E}$ for the *n*-butanol and *n*-pentanol systems were much like those seen with the methanol and ethanol systems, i.e., the P_T behavior for TEAB with full thickness skin was 'sigmoidal' and the epidermis/dermis be-

haved much like a porous membrane over the alkanol concentration range investigated.

Calculation of the enhancement factor, E, for E2β and HC in the various alkanol / saline systems

The transport enhancement of the lipoidal pathway induced by the alkanols for E2β and HC as permeants may be determined as previously described using the following equation which is readily obtained by combining Eqns 6–8,

$$E = (P_{L,X}/P_{L,O})(S_X/S_O) \qquad (9)$$

$P_{L,X}$ and $P_{L,O}$ may be calculated from data given in Figs 2–7 and Eqns 1 and 2 using the appropriate P_T and $P_{D/E}$ values and the P_P value taken from the TEAB experiments (for which $P_T \approx P_{SC} \approx P_P$). The solubility data for E2β and HC used in the calculations are given in Fig. 8.

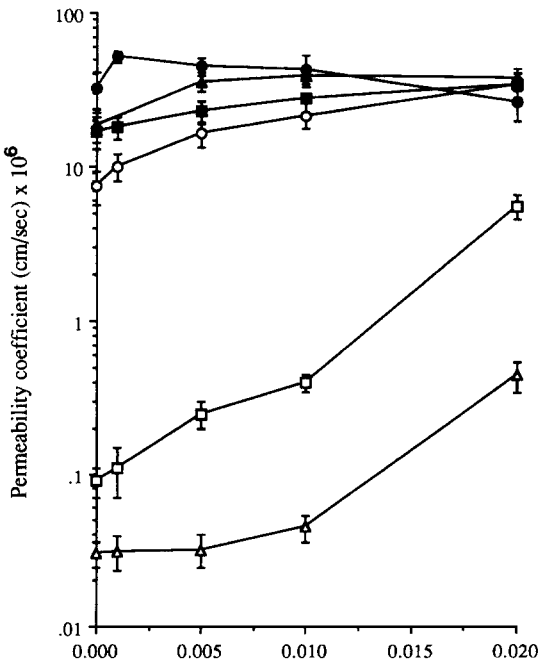

Fig. 7. Permeability coefficients of whole skin (open symbols) and stripped skin (closed symbols) as a function of volume fraction of *n*-pentanol (*n*-PtOH)/saline for β-estradiol (circles), hydrocortisone (squares) and TEAB (triangles), respectively. Each data point represents the mean and standard deviation of four determinations.

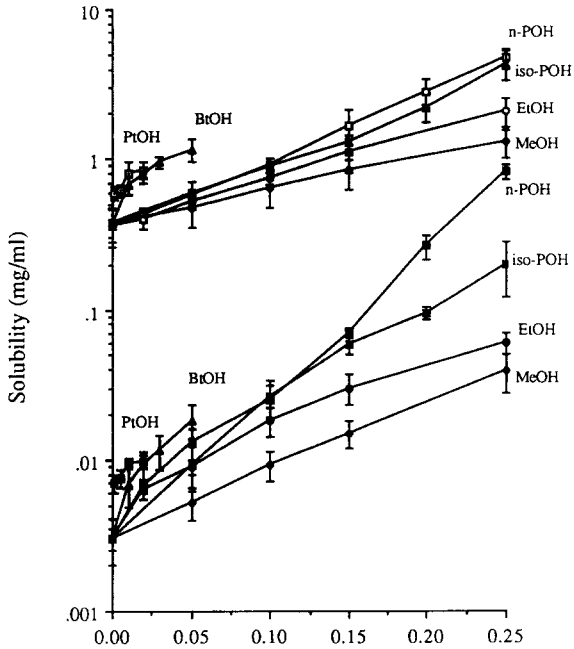

Fig. 8. Solubilities of β-estradiol (closed symbols) and hydrocortisone (open symbols) in different volume fractions of *n*-alkanol/saline. Each data point represents the mean and standard deviation of four determinations.

The E values for E2β and HC calculated in this way are presented in Fig. 9 for the *n*-alkanol/saline systems as a function of the alkanol concentration. For Fig. 9, some judgment was used with regard to the selection of the highest alkanol concentrations for the E value calculations, based on whether meaningful E values could be obtained by Eqns 1, 2, and 9. For example, for the ethanol and methanol systems, it was felt that new pore induction was modest below alkanol levels of 0.5 and therefore the E values calculated below 0.5 were believed to be acceptable. It is seen from Fig. 3 that, at an ethanol concentration of 0.75, P_P of TEAB is greater than the P_T values for both E2β and HC and negative P_L values would result from Eqn 2. A similar problem exists for both the *n*-propanol and isopropanol systems: the calculations for the

E values above 0.25 would be questionable. Another kind of a problem occurs with the n-butanol and n-pentanol systems. This is that, at the higher alkanol concentrations (~ 0.03 for n-butanol and ~ 0.02 for n-pentanol), the P_T values for E2β become very close to the $P_{D/E}$ values and errors in the calculation of P_{SC} using Eqn 1 become very large. It is believed safe to state that for purposes of quantitative discussion, E values of around 10 or less are most likely to be reliable and, in much of the analysis to follow, we shall restrict comments to the low alkanol concentration regions of Fig. 9.

Two points stand out from Fig. 9. First, the E values are essentially the same for E2β and HC. This was noted previously (Ghanem et al., 1992) in the study with three lipophilic permeants in the ethanol/saline systems. We see now that this similarity of E values for E2β and HC is probably true for all of the n-alkanol/saline systems investigated here. Whether the E values will be the same (or nearly the same) for all permeants

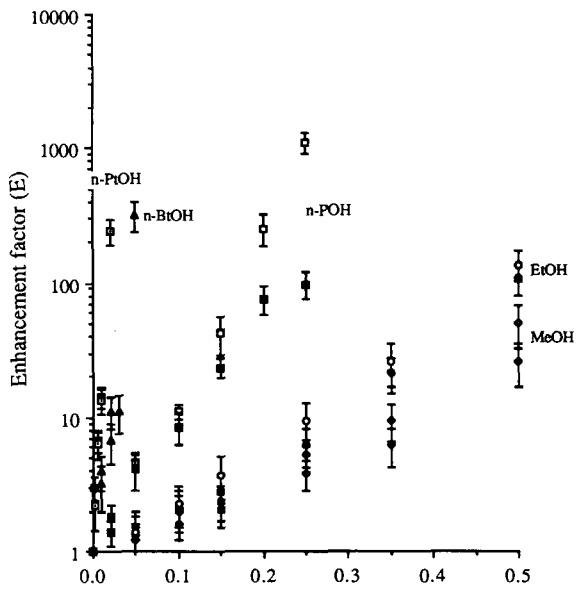

Fig. 9. Mean and standard deviation of the enhancement factor, E, as a function of volume fraction of n-alkanol/saline for β-estradiol (closed symbols) and hydrocortisone (open symbols).

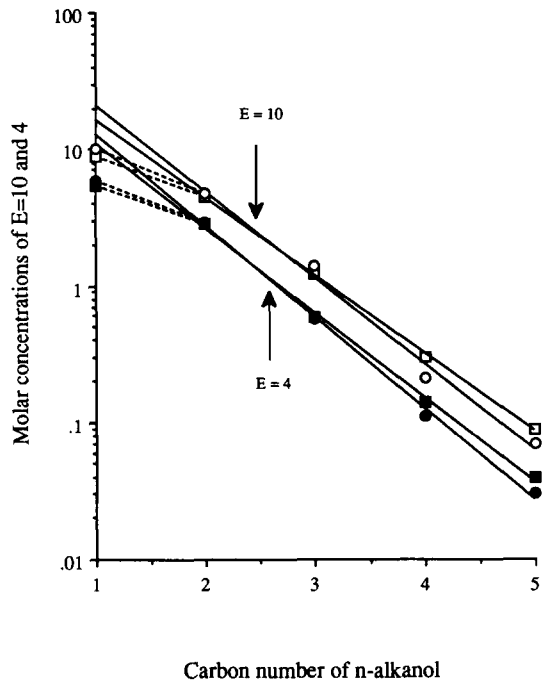

Fig. 10. Relationship between the carbon number of n-alkanol and the molar concentrations of n-alkanol which give an enhancement factor, E, of 10 and 4 for β-estradiol (circles) and hydrocortisone (squares).

transported via the lipoidal pathway remains to be seen. Perhaps, this constancy of enhancement behavior would depend importantly upon the molecular sizes of the compared permeants being approximately the same. Liu (1989) has shown that ethanol may enhance the transport of ethanol across hairless mouse skin to a significantly lesser extent than the steroids. Also, Lambert et al. (1989) showed that Azone at the same dose was able to enhance the transport of steroids across hairless mouse skin to a much greater extent than the transport of ethanol or butanol. The second major point with regard to the data in Fig. 9 is that there is a systematic alkanol chain length effect upon E. The relationship between the chain length and E is seen more clearly in Fig. 10 where the logarithms of the alkanol concentrations at $E = 10$ and $E = 4$ are plotted against the carbon number of the n-alkanol. These 'iso-enhancement' concentrations (at $E = 10$ and $E = 4$) are monotonically related to the carbon number of the n-alkanol; if methanol is excluded, there is

TABLE 1

Slopes of semi-log plots between the carbon number and the molar concentrations of n-alkanol which give enhancement factors, E, of 10 and 4 for β-estradiol (E2β) and hydrocortisone (HC)

		Based on C2–C5	Based on C3–C5
$E = 10$	E2β	−0.630	−0.649
	HC	−0.570	−0.570
$E = 4$	E2β	−0.667	−0.639
	HC	−0.616	−0.584
	Average	-0.621 ± 0.04	-0.611 ± 0.04

an approximately linear relationship between the logarithm of the iso-enhancement concentration and the carbon number with a slope of about −0.61 (see Table 1). A possible mechanistic interpretation of this chain length effect upon E is discussed later.

E values for E2β and HC in the isopropanol / saline system

The E values for the isopropanol/saline system were calculated in the same manner as above using the data given in Fig. 5 and are presented in Fig. 11. For comparison, the *n*-propanol/saline results are also shown in Fig. 11. Again, as was generally true in the *n*-alkanol/saline systems, the E values for E2β and HC in the isopropanol/saline system are nearly the same at the low alkanol concentrations.

A finding in this study is that *n*-propanol is a more potent enhancer (by around a factor of 2) of the lipoidal pathway than isopropanol. Interestingly, Friend et al. (1988) found that neat *n*-propanol as the donor phase is about twice as effective as neat isopropanol in the transport of levonorgestrel across rat skin in vitro. Although the findings are similar, we hesitate to compare the two sets of studies because, in our experiments, the solvent compositions were the same in both the donor and receiver chambers; this was not the case in the studies of Friend et al.

A possible interpretation of the alkyl chain length effect upon E for the n-alkanols

In the following, an attempt will be made to analyze the alkyl chain length effect. It is seen

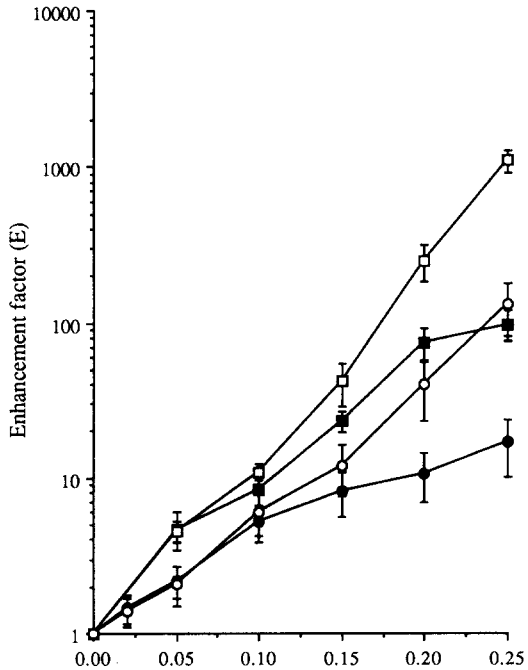

Fig. 11. Comparison between the enhancement factor, E, of isopropanol (circles) and *n*-propanol (squares) for β-estradiol (closed symbols) and hydrocortisone (open symbols).

from Table 1 that the slope of the linear portions of the plots in Fig. 10 is -0.61 ± 0.04. This means that, when the *n*-alkanol chain is increased by one methylene group, the logarithm of the E value is increased by 0.61 ± 0.04 and the E value itself is increased by a factor of $\Delta E = 4.1 \pm 0.4$.

ΔE may be interpreted as being comprised of two factors, $\Delta E = \Delta E_1 \times \Delta E_2$, where ΔE_1 is the increase in the partitioning tendency of the *n*-alkanol from the bulk solution phase into the rate-limiting microenvironment and where ΔE_2 is the increase in the 'intrinsic' potency of the *n*-alkanol as an enhancer for the lipoidal pathway. It is suggested that an estimate of ΔE_1 may be obtained from appropriate permeability coefficient data: when the alkyl chain length for a set of permeants is varied in the transport experiments, the methylene group effect upon the partitioning tendency may be deduced (the effect of a methy-

lene group upon the diffusivity is assumed to be negligible here). Durrheim et al. (1980) found ~ 0.26 as the logarithm of the methylene group effect for hairless mouse skin at 37°C with *n*-alkanol permeability coefficient up to *n*-octanol. Scheuplein and Blank (1971) obtained a value of 0.33 for human stratum corneum with the *n*-alkanols as permeants at 25°C. With hydrocortisone 21-esters, Smith (1982) found a value of 0.22 for hairless mouse skin at 37°C. Most recently, Raykar (1985) determined a value of 0.275 for the methylene group effect in human stratum corneum at 37°C also with hydrocortisone 21-esters. The average of the above values is 0.27 ± 0.05. We may thus propose that $\log(\Delta E_1) \approx 0.27$; therefore, $\log(\Delta E_2) \approx 0.61 - 0.27 \approx 0.34$, and the methylene group contribution to the intrinsic enhancer potency for the *n*-alkanols (from C_3 to C_5) is $\Delta E_2 \approx 2.2$.

The above analysis leaves the reader with several unanswered questions. Most important of these, perhaps, relates to the assumption that the methylene group contribution to the partitioning tendency for a permeant deduced from permeability coefficients is the same as that for the *n*-alkanol when it plays the role of the enhancer. It is conceivable that the relevant microenvironments may be different in the two situations. Another question may be whether the situations for $E = 10$ and/or $E = 4$ correspond to sufficiently low *n*-alkanol concentrations so that Henry's law can be assumed for both the solution and the rate-limiting microenvironment of the stratum corneum lipid phase(s). More work needs to be done before the concept of ΔE_1 and ΔE_2 and the values deduced for them (above) may be considered to be sound and meaningful. Nevertheless, the above analysis is useful in providing an insight into the possible mode of action of the *n*-alkanols as permeation enhancers.

The effects of pretreating hairless mouse skin with aqueous n-alkanol solutions

Previously, we had shown that even up to 50% ethanol, 2 h exposures of hairless mouse skin to aqueous ethanol elicited no irreversible effects upon the permeation of E2β (Ghanem et al., 1987b) in saline experiments. It was felt, however,

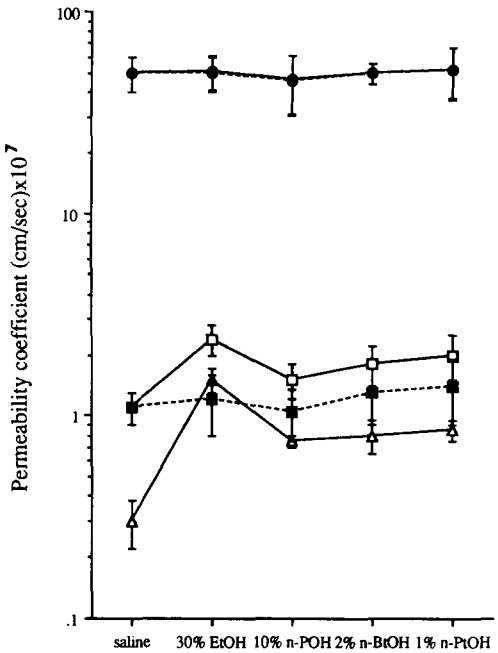

Fig. 12. Effect of pretreatment of hairless mouse skin with different volume fractions of *n*-alkanol/saline under iso-enhancement conditions for 4 h on the permeability coefficients of β-estradiol (circles), hydrocortisone (squares), and TEAB (triangles) in saline in both compartments. Closed symbols for β-estradiol and hydrocortisone represent the values after correction for the pore pathway increase (TEAB results, open triangles) by pretreatment. Each data point is the mean and standard deviation of four determinations.

that E2β (being a highly lipophilic permeant) would be sensitive only to changes in the lipoidal pathway and would not be sensitive to moderate changes in the pore pathway. In order to gain an understanding of the possible irreversible effects of exposing hairless mouse skin to the various *n*-alkanol situations, a limited study of pretreatment effects with the aqueous *n*-alkanols was carried out; E2β, HC, and TEAB were used as the probe permeants so that both the lipoidal and pore pathways could be assessed.

Fig. 12 summarizes the results of this study. Hairless mouse skin was pretreated for 4 h in 30% ethanol, 10% *n*-propanol, 2% *n*-butanol, or 1% *n*-pentanol. These alkanol concentrations are all close to the iso-enhancement concentrations

for $E = 10$. The main findings are as follows. With E2β as the probe permeant, there were no irreversible effects; this is consistent with our earlier study (Ghanem et al., 1987b). With both HC and TEAB as the probe permeants, some irreversible effects can be noted; in both instances, the permeability coefficients after pretreatment were always larger than those before pretreatment and the effects were always greater (percentagewise) for TEAB than for HC. An important outcome of this study is that, when the HC data are corrected for the pore pathway changes (using Eqn 2 and the TEAB data after pretreatment), the values of the HC permeability coefficients return to the original saline value. The results presented in Fig. 12, therefore, strongly support the view that the pretreatments caused increases in P_P of around a factor of 2 to 3; importantly, however, there appears to have been no significant effects of the pretreatment exposures upon P_L. It is believed, therefore, that the P_L values deduced for both E2β and HC from the experimental P_T values using Eqns 1 and 2 are likely to be 'good' values and not compromised by possible damage caused by the exposure to the *n*-alkanols. It should be worthwhile to point out that the results presented in Fig. 12 also subtly, but strongly, support the validity of the model itself (depicted in Fig. 1 and quantified by Eqns 1 and 2).

Although not related to the main point of this discussion, Table 2 compares the TEAB P_T data obtained directly in the *n*-alkanol solutions with those measured in saline after pretreatment in the same *n*-alkanol solutions. These results show that, except for 10% *n*-propanol, the P_T values after the *n*-alkanol pretreatment seem to be always greater than those obtained directly in the *n*-alkanol solutions. These findings therefore suggest that the two-step protocol in the pretreatment studies may cause somewhat greater pore pathway damage than running the experiment directly in the *n*-alkanol media. One must be cautious in comparing the two sets of data in this way because we do not have actual diffusivities of TEAB in these *n*-alkanol solutions and they may differ significantly from the diffusivity value in saline in some instances; for example, the viscosity of 30% ethanol is about double that of saline and this may reduce the difference between the permeability coefficients with pretreatment (1.50) and without pretreatment (0.40) for the 30% ethanol case in Table 2.

TABLE 2

Effect of pretreatment on the permeability coefficient of hairless mouse skin for TEAB under iso-enhancement conditions

Vehicle	P (cm/s) ($\times 10^7$) $\pm$ S.D. ($n = 4$)	
	Without pretreatment	With pretreatment
Saline	0.30 $\pm$ 0.01	0.30 $\pm$ 0.01
30% ethanol [a]	0.40 $\pm$ 0.01	1.50 $\pm$ 0.20
10% *n*-propanol [a]	0.84 $\pm$ 0.10	0.75 $\pm$ 0.05
2% *n*-butanol [a]	0.45 $\pm$ 0.10	0.80 $\pm$ 0.15
1% *n*-pentanol [a]	0.45 $\pm$ 0.01	0.85 $\pm$ 0.10

[a] Iso-enhancement concentrations of *n*-alkanols ($E = 10$).

Finally, it should be appropriate to relate the conclusions of these pretreatment experiments to the recent publication of Kai et al. (1990), who came to the conclusion that "the principal mechanism by which the alkanols enhance percutaneous absorption is suggested as being extraction of stratum corneum intercellular lipids". Based upon the above findings (i.e., that there appear to be *no irreversible effects* on the transport behavior of E2β and HC after the P_P corrections are made), it is concluded that, at the lower *n*-alkanol concentrations (see Fig. 12), lipid extraction *is not* the principal mechanism by which *n*-alkanols enhance percutaneous absorption for the lipoidal pathway. In the present investigation, there appears to have been little or no lipid extraction up to E values of around 10 that had direct bearing upon transport via the lipoidal pathway; otherwise, the results presented in Fig. 12 could not have been obtained.

At very high alkanol concentrations, however, there may well have been significant lipid extraction relevant to transport. It was pointed out previously (Ghanem et al., 1987b, 1992) and again in the present study that, at high ethanol concentrations ($\geq 75\%$), there was extensive new pore formation in hairless mouse stratum corneum, and one explanation for this may be lipid extraction by the solvent. However, there is a caveat

here: Grubauer et al. (1989) and Smith and Anderson (personal communication) found that the highly nonpolar organic or hydrocarbon solvent treatment of human stratum corneum removes a substantial percentage of the total lipids with little or no change in permeability of the stratum corneum; the barrier property of the stratum corneum is not simply/directly related to lipid extraction.

A test of the applicability of Henry's law for E2β and HC in the n-alkanol solutions

It is implicit in the present approach that Henry's law is obeyed for E2β and HC in all of the *n*-alkanol solutions up to their solubility concentrations. If Henry's law is not obeyed, Eqn 9 should not be used in the calculation of the E values, and another method would be needed for obtaining the hypothetical partition coefficient ratio, $(K_O/K_X)_S$. In a previous study (Ghanem et al., 1992), $(K_O/K_X)_S$ was obtained by conducting actual partitioning experiments using hexadecane as the oil phase paired with saline and with ethanol/saline at low permeant concentrations so that Henry's law would be obeyed. The approach of using hexadecane was not considered in the present study because of the likely extensive transfer of the higher alkanols into the hexadecane phase from the aqueous phase.

The approach taken in the present work was an indirect one and simply involved comparing P_T values obtained at tracer level concentrations with those measured at solubility saturation. Table 3 summarizes the findings. As can be seen, in all cases (including $E = 10$ iso-enhancement concentrations for the *n*-alkanols), the P_T values obtained at saturation and at tracer levels are the same within experimental error. It is therefore concluded that Henry's law is likely obeyed in all instances.

The present approach is not a perfect one, because one may argue that, as an example, the following situation may occur. The tracer level and the saturation P_T values may be nearly the same but this may be accidental, arising from a cancellation of two effects: (a) a deviation from Henry's law because of solute-solute association and (b) enhanced permeation caused by the permeant itself acting as an enhancer at high concentrations. It is believed that such situations (involving exact cancellation of the effects) are unlikely and therefore probably do not apply to the results in Table 3; the likelihood of Henry's law not being obeyed should be very small.

TABLE 3

Permeability coefficients of hairless mouse skin for β-estradiol (E2β) and hydrocortisone (HC) at tracer and saturation levels

Vehicle	v/v %	P of E2β (cm/s) ($\times 10^6$)		P of HC (cm/s) ($\times 10^7$)	
		Tracer [b]	Saturation [c]	Tracer [b]	Saturation [c]
Saline	100	7.5±1.9	7.3±1.2	1.1±0.3	1.3±0.2
Ethanol	30 [a]	2.1±0.6	2.2±0.3	1.5±0.3	1.6±0.2
n-Propanol	25	5.0±1.4	5.1±1.0		
	10 [a]	5.6±1.9	7.3±1.4	3.3±0.6	3.2±0.4
n-Butanol	5	20.0±6.5	24.1±5.5		
	3	17.5±4.5	14.0±1.4		
	2 [a]	17.1±4.8	14.0±2.5	2.4±0.7	2.9±0.6
n-Pentanol	2	34.8±4.1	29.2±3.8		
	1 [a]	21.7±4.0	20.0±2.8	4.0±0.5	4.7±0.8

[a] Iso-enhancement concentrations of *n*-alkanols ($E = 10$).
[b] Mean ± S.D. ($n = 4$).
[c] Mean ± S.D. ($n = 3$).

Possible mechanism for the enhancer action of the n-alkanols

First, the evidence and arguments presented are compelling that at the low *n*-alkanol levels (i.e., $E \approx 10$), there is little or no irreversible change associated with the lipoidal pathway. The situation would be quite different if the *n*-alkanol concentrations were much greater (Ghanem et al., 1987b; Kai et al., 1990). For the discussion to follow, we are concerned with the situations at $E = 10$ or less.

Fourier transform infrared (FTIR) studies (Krill, 1989) of the effects of short chain alcohols on the lipid alkyl chain packing, mobility, and conformational order at physiologic temperature (37°C) in hairless mouse stratum corneum have provided no evidence for gross lipid alkyl chain fluidization at aqueous alkanol concentrations corresponding to E values of around 10 (Fig. 9). The data therefore suggest that the simple idea

of the *n*-alkanols causing gross biomembrane fluidity increases as might be expected of a lipid bilayer fluidizing agent, such as suggested for oleic acid (Golden et al., 1986; Goodman and Barry, 1989), may not explain the mechanism of action of the alkanols as far as the transport enhancement of the lipoidal pathway is concerned. A possible mode of action of short chain alkanols in the enhancement of the lipoidal pathway for permeation may involve the polar head region of the bilayer or the region slightly below this polar head plane or both. Anderson and Raykar (1989) have pointed out that permeant functional group contributions to permeant transport across stratum corneum are most consistent with the rate-limiting microenvironment being significantly more polar than would be the case for pure hydrocarbon. Also, fluorescence probe studies (Kim et al., 1990) with and without added *n*-alkanols support this idea: large changes in fluorescence polarization are observed with probes believed to be positioned at intermediate depths (C2–C9) in the stratum corneum lipid liposomes (Wertz et al., 1986), but not in the deep (C16) reaches of the bilayer, thus suggesting the intermediate depths as being the primary action sites for the short chain alkanols. Short chain alkanols may solvate the lipid-water interface, intercalating and disrupting the interactions of the upper regions of alkyl chains and those between the polar head groups. This could result in an increase in the effective interfacial area of the bilayer and a likely increase in both diffusivity and partitioning tendency for a permeant in this microenvironment (and thereby contributing to making both D_X/D_O and F greater than unity).

Acknowledgments

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