Cotransport of Estradiol and Ethanol Through Human Skin *in Vitro*: Understanding the Permeant/Enhancer Flux Relationship

Puchun Liu,[1] Tamie Kurihara-Bergstrom,[1,2] and William R. Good[1]

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The thermodynamic and kinetic limits of ethanol-enhanced estradiol skin transport have been investigated by studying the relationship between estradiol and ethanol steady-state flux in the cotransport of permeant and enhancer in situations in which there exists an enhancer solvent gradient across the skin ("asymmetric" configuration). For aqueous ethanol solution saturated with estradiol, the flux of estradiol across the human epidermal membrane is empirically observed to be linear with the ethanol flux. A physical model approach has been used to determine the basis of this empirical linearity and to predict permeant/enhancer transport across the skin for the asymmetric configuration. Enhancement factors, determined with a balanced ethanol concentration across the skin ("symmetric" configurations), are used to predict fluxes in the asymmetric configurations. The model demonstrates that ethanol enhances the stratum corneum transport of estradiol and of itself by increasing the respective diffusion coefficients at lower concentrations (<50%) and by both increasing the diffusion coefficients and decreasing the membrane activity coefficients at moderate concentrations (50 to 75%). The model also demonstrates that the permeant flux, in general, is not linear with the cotransported enhancer flux.

KEY WORDS: ethanol; estradiol; human skin transport; enhancement factor; permeant/enhancer flux relationship; "symmetric"/"asymmetric" physical models.

INTRODUCTION

Estraderm, an estradiol transdermal device, consists of an ethanol-based drug reservoir with a rate-controlling membrane. Use of ethanol as a solvent for estradiol provides a sufficiently large gradient of estradiol across the skin to result in estradiol flux in a useful range (1). Ethanol and other short-chain alkanols have been found to enhance skin mass transport of estradiol and other permeants (2–4). It has been hypothesized in recent diffusional and spectroscopic studies that aqueous ethanol reduces stratum corneum lipid polar head interactions (3) and alters stratum corneum's keratinized protein conformation (4). Further mechanistic studies on influences of ethanol in estradiol skin permeation have been undertaken in "asymmetric" configuration, where aqueous ethanol is present in the donor solution and water only in the receiver solution so that there is significant cotransport of the enhancer solvent along with the principal permeant (5–7). As shown here, the flux of estradiol across human cadaver epidermis is observed to be linearly correlated with the ethanol flux. Such a linear dependence of the solvent enhancer has also been observed for other permeant/enhancer situations such as nitroglycerin/ethanol (8) and levonorgestrel/ethyl acetate-ethanol (9). Mechanistically understanding this empirical linearity would be valuable from both a theoretical and a practical standpoint.

The purpose of this study is, with a physical model approach, to address fundamental questions including (a) What is the physical basis of this empirically linear correlation on the flux of estradiol and of ethanol? and (b) How may this be applied to other permeant/enhancer systems? The thermodynamic and kinetic parameters controlling both estradiol and ethanol permeation are quantified with *in vitro* experiments using human cadaver skin. More specifically, the enhancement factors determined in the "symmetric" experiments are used to predict the flux in the asymmetric experiments and to understand the permeant/enhancer flux relationship.

THEORETICAL CONSIDERATION

For the "symmetric" configuration with a homogeneous membrane in which the membrane (subscript m)-to-vehicle (subscript v) partition coefficient (K) is written as the ratio of activity coefficients in the vehicle, γ_v, and in the membrane, γ_m (10),

$$K = \frac{\gamma_v}{\gamma_m} \tag{1}$$

Fick's first law, with a constant activity in the donor chamber and an effective sink condition in the receiver chamber, may be expressed as

$$\frac{D}{h\gamma_m} = \frac{P}{\gamma_v} = \frac{J}{a_d} \tag{2}$$

where J, P, D, and a are the steady-state flux, the permeability coefficient, the diffusion coefficient, and the activity, respectively; h is the diffusion path length in the membrane, and subscript d refers to the donor solution or to the membrane surface in the donor side. Therefore, the enhancement factor (E) may be defined by considering the membrane-related properties $[D/(h\gamma_m)]$ at infinitely diluted enhancer concentration (superscript o) as a reference state and measuring any change of the membrane-related properties when the significant enhancer activity (superscript asterisk) is applied.

$$E = \frac{\left(\frac{D}{h\gamma_m}\right)^*}{\left(\frac{D}{h\gamma_m}\right)^o} = \frac{\left(\frac{P}{\gamma_v}\right)^*}{\left(\frac{P}{\gamma_v}\right)^o} = \frac{\left(\frac{J}{a_d}\right)^*}{\left(\frac{J}{a_d}\right)^o} \tag{3}$$

In the case of cotransport of permeant and enhancer, both permeant and enhancer itself are enhanced. For the enhancer (subscript E), the enhancement factor is determined with Eq. (3). However, since the saturated permeant (subscript P) in the donor chamber gives the same permeant activity in the different enhancer concentration, the en-

[1] Pharmaceuticals Division, CIBA-GEIGY Corporation, Ardsley, New York 10502.

[2] To whom correspondence should be addressed.

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hancement factor for the saturated permeant (E_p) may be simply determined as a ratio of the flux:

$$E_P = \frac{J_P^*}{J_P^o} \tag{4}$$

The concept developed above is for simple membranes; the underlying principles are general, however, and can be applied to the stratum corneum. In the cases (low to moderate ethanol concentration) with a relatively unchanged diffusion path length of stratum corneum lipoidal pathway, total enhancement factor [Eq. (3)] may be mechanistically described with two simple theoretic limits: the kinetic model ($E = D^*/D^o$ with constant γ_m), where the lipoidal pathway is physically altered (e.g., structural disorder) in the presence of the enhancer, and the thermodynamic model ($E = \gamma_m{}^o/\gamma_m{}^*$ with constant D), where the chemical composition (e.g., polarity) of lipoidal barrier region is altered by the enhancer.

In the "asymmetric" configuration (superscript $^{*/o}$) with enhancer gradient across the membrane, the steady-state flux equation is modified (6,7), where both the diffusion coefficient ($D^{*/o}$) and the activity coefficient ($\gamma_m{}^{*/o}$) are position (x)-dependent across the membrane.

$$J^{*/o} = -D^{*/o}\frac{dC_m}{dx} \tag{5}$$

where the concentration within the membrane (C_m) is given by

$$C_m = \frac{a}{\gamma_m} \tag{6}$$

Integration of Eq. (5) is shown in the Appendix, resulting in the asymmetric fluxes and the permeant/enhancer flux ratio. Table I summarizes simple equations of the asymmetric flux and of the permeant/enhancer flux ratio for several simple theoretic limits including the kinetic model, the thermodynamic model, and the simplified general model. It is clearly demonstrated that (a) the asymmetric fluxes may be predicted with the ethanol activity and the enhancement factor and (b) the saturated permeant flux is not linearly correlated with the enhancer flux in general.

MATERIALS AND METHODS

Materials

β-Estradiol was obtained commercially (Diosynth, Netherlands). Absolute ethanol (USI, Tuscola, IL) was used to prepare aqueous ethanol solutions. HPLC-grade acetonitrile (J. T. Baker Inc., Phillipsburg, NJ) was used as received. Human cadaver skin was obtained from a skin bank. The epidermal membrane (stratum corneum + viable epidermis) was isolated by the heat separation technique (11).

Skin Permeation Experiments

Skin permeation experiments were carried out in two different configurations for various ethanol concentrations: (a) "asymmetric" configurations, where ethanol solution is present in the donor chamber only and water in the receiver chamber, the fluxes of estradiol and ethanol being simultaneously determined; and (b) "symmetric" configurations, where the same aqueous ethanol solution is present in both chambers and only estradiol flux is determined. For estradiol experiments, the two-chamber diffusion cells and experimental procedures are similar to those reported earlier (4). The samples were diluted and centrifuged prior to high-performance liquid chromatography (HPLC) and gas chromatography (GC) assays. The symmetric experiment of ^{14}C-ethanol has been reported and is used to determine the influence of symmetric ethanol on itself (12,13). For both estradiol and ethanol, the flux, J (for the asymmetric configurations), and the permeability coefficients, P (for the symmetric configurations), were calculated by

$$P = \frac{J}{C_D} = \frac{(dC_R/dt)(V_R/A)}{C_D}$$

where V_R is the receiver volume, A is the effective diffusional area, C_D and C_R are the donor and receiver chamber concentrations, respectively, and dC_R/dt is the steady-state slope of the receiver concentration-versus-time curve.

Table I. Summary of Simple Equations for Kinetic Model, Thermodynamic Model, and Simplified General Model[a]

	Kinetic model		Thermodynamic model		Simple general model	
Enhancer flux	$J_E^{*/o} = \frac{P_E^o}{\gamma_{v,E}^o}\int_0^{a_{E,d}^{*/o}} E_E^{*/o}\,da_E$	(K1)	$J_E^{*/o} = \frac{P_E^o}{\gamma_{v,E}^o} E_{E,d}^{*/o}\, a_{E,d}^{*/o}$	(T1)		
Permeant flux	$J_P^{*/o} = J_P^o \frac{\int_0^{a_{E,d}^{*/o}} E_E^{*/o}\,da_E}{\int_o^{a_{E,d}^{*/o}} E_E^{*/o}/E_P^{*/o}\,da_E}$	(K2)	$J_P^{*/o} = J_P^o\, E_{P,d}^{*/o}$	(T2)		
Permeant/enhancer flux ratio	$\frac{J_P^{*/o}}{J_E^{*/o}} = \frac{J_P^o}{P_E^o}\frac{1}{\int_0^{a_{E,d}^{*/o}} (E_E^{*/o}/E_P^{*/o})\,da_E}$	(K3)	$\frac{J_P^{*/o}}{J_E^{*/o}} = \frac{J_P^o}{P_E^o}\frac{1}{(E_{E,d}^{*/o}/E_{P,d}^{*/o})a_{E,d}^{*/o}}$	(T3)	$\frac{J_P^{*/o}}{J_E^{*/o}} = \frac{J_P^o}{P_E^o}\frac{1}{a_{E,d}^{*/o}}$	(G3)

[a] Kinetic Model: $E = D^*/D^o$ with constant γ_m.
Thermodynamic Model: $E = \gamma_m{}^o/\gamma_m{}^*$ with constant D.
Simple General Model: at any point in the membrane, γ_{mP} is proportional to γ_{mE} and D_P is proportional to D_E.

HPLC and GC Analytical Methods

Estradiol was assayed by reverse-phase HPLC (WISP Model 710 with Model 510 programmable solvent pump, Waters, Milford, MA; Spectroflow 980 programmable fluorescence detector, KRATO Analytical Instruments, Bristol, CT). A C-18 column (Perkin-Elmer, Cupertino, CA) with a mobile phase of acetonitrile–water (40:60) was used. Sample detection was accomplished by using an excitation wavelength of 228 nm and no emission filter. The flow rate was 1 ml/min, the injection volume was 10 μl, and the retention time was 1.0 min. Ethanol was quantitated by GC (4).

RESULTS

The experimental fluxes of estradiol and ethanol in the "asymmetric" configuration are presented up to 75% ethanol concentration (Table II). Twenty experiments were conducted with human epidermal membrane from three skin donors. The flux varies considerably from skin donor to skin donor (about 75%) and within the same skin donors (about 30%). As shown in Table I, in spite of the variations, an increase in the flux for both estradiol and ethanol was observed with increasing ethanol concentration.

Figure 1 presents the flux of estradiol from saturated solution with a wide range of ethanol concentrations (0 to 75%) in "symmetric" configuration. Estradiol flux increases with increasing ethanol concentration, indicating the ethanol enhanced estradiol transport [Eq. (4)]. The permeability coefficients of ^{14}C-ethanol, obtained in the previous reports (12,13), are summarized in Fig. 2.

Partial vapor pressure data (14) were used to estimate ethanol activity coefficient in the aqueous ethanol solutions. As shown in Fig. 3, the relative activity coefficient of ethanol decreases with increasing ethanol concentration. Both the increase in the permeability coefficient and the decrease in the activity coefficient will contribute to the self-enhancement factor for ethanol [Eq. (3)]. The ethanol activity is also plotted in Fig. 3. The increment of ethanol activity decreases with increasing concentration.

DISCUSSION

"Asymmetric" Fluxes: Comparison of Data with Model Predictions

The enhancement factors, determined from Eqs. (3) and (4), are used in predictions of asymmetric fluxes. Figure 4 summarizes the enhancement factor as a function of ethanol activity for both estradiol and ethanol. The results are consistent with previously reported data (2,12,13) demonstrating that ethanol significantly enhances the lipoidal pathway of hairless mouse stratum corneum and human stratum corneum for estradiol and ethanol itself. As seen in Fig. 4, increasing ethanol activity results in a dramatical increase in enhancement factor for both estradiol and ethanol.

With the simple equations in Table I, the theoretical fluxes may be calculated for the two theoretical limits: ki-

Table II. Cotransported Flux of Saturated Estradiol and Ethanol Through Human Epidermal Membrane as a Function of Ethanol Concentration in Donor Chamber ("Asymmetric" Configuration)

Ethanol (v%) in the donor	Steady-state flux (μg/hr/cm^2)[a]		Skin donors[b]	No. of expts.
	Estradiol	Ethanol		
0	0.0314 ± 0.00788		ABC	6
25	0.108 ± 0.0221	584 ± 178	A C	5
35	0.203 ± 0.0756	760 ± 35.0	A	3
50	0.165 ± 0.0242	952 ± 5.66	C	2
75	0.348 ± 0.183	1853 ± 668	ABC	6

[a] The experiments were conducted with "infinite dose" for 24 hr. The lag times are about 3 and 0.3 hr for estradiol and ethanol, respectively. The fluxes are expressed as average ± standard deviation.

[b] The three different skin donors are represented as A, B, and C.

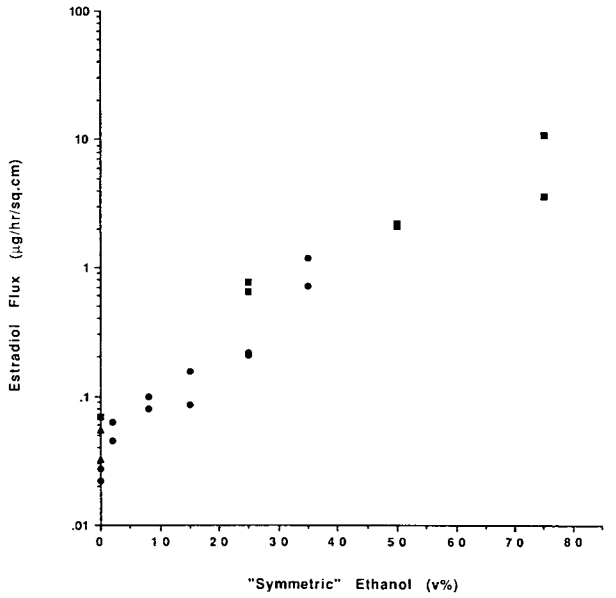

Fig. 1. Flux of saturated estradiol in human epidermal membrane as a function of ethanol concentration on both sides of the skin ("symmetric" configuration). The different symbols indicate different skin donors.

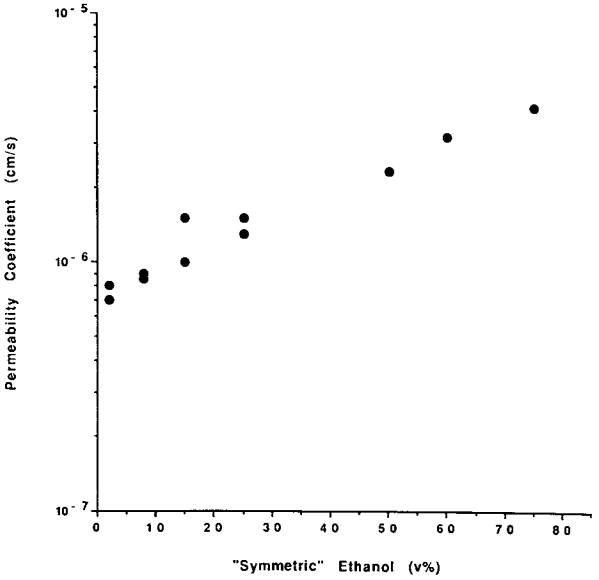

Fig. 2. Permeability coefficient of ^{14}C-ethanol with human epidermal membrane as a function of ethanol concentration on both sides of the skin ("symmetric" configuration).

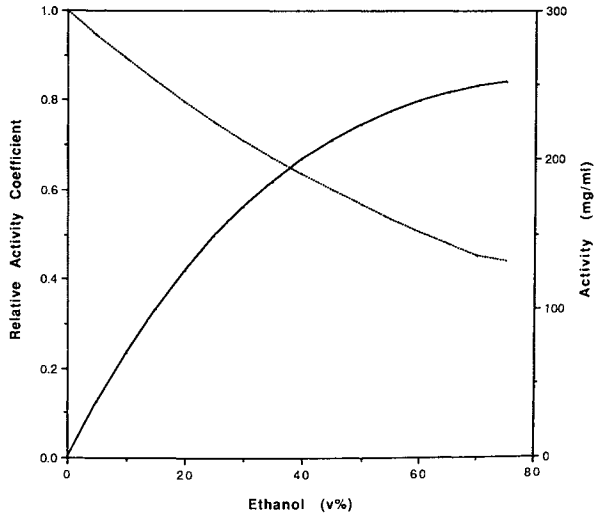

Fig. 3. Ethanol activity coefficient (dashed line) and ethanol activity (solid line), calculated from ethanol vapor pressure data, as a function of ethanol concentration.

netic model [Eqs. (K1) and (K2)] and thermodynamic model [Eqs. (T1) and T2)]. The kinetic model ($E = D^*/D^\circ$ with constant γ_m) describes the limiting situation where the lipoidal pathway is physically altered (e.g., an increase in structural disorder) in the presence of the enhancer and the thermodynamic model ($E = \gamma_m^\circ/\gamma_m^*$ with constant D) describes the limiting situation where the chemical composition of the lipid barrier region is altered by the enhancer (e.g., significant enhancer uptake by the barrier). For the kinetic model, the fluxes account for the gradients of enhancer activity and of enhancement factors across the stratum corneum. For the thermodynamic model, however, the properties (the enhancer activity and the enhancement factors) in the donor solution are enough to specify the asymmetric diffusion system and the fluxes are expected to be very sensitive to changes of the enhancer concentration in the donor solution. The experimental data and the predicted fluxes with these two models are compared for both estradiol and ethanol in Fig. 5. Over a wide range of conditions, these data clearly demonstrate the failure of the thermodynamic model to fit the experimental results. In consideration of the donor-to-donor variations, the kinetic model better represents the trend of ethanol and estradiol fluxes. However, the deviation of the kinetic model from the experimental fluxes at moderate ethanol donor concentrations (50 to 75%, especially for estradiol) may suggest that there is a departure from the simple diffusion-determined trend. That is, with increasing ethanol concentration, the thermodynamic model appears to have some contribution to the overall skin transport, especially for the permeant, estradiol. A combined model using both kinetic and thermodynamic models may best fit the experimental results at moderate ethanol donor concentrations.

Based on the previously reported results with stratum corneum (2,3,12,15,16) and other biological membranes (17), an ethanol concentration-dependent enhancement mechanism may be proposed. First, at lower ethanol levels, ethanol may interact at the surface of the lipid polar head so that the lipoidal barrier region (e.g., the hydrocarbon-chain re-

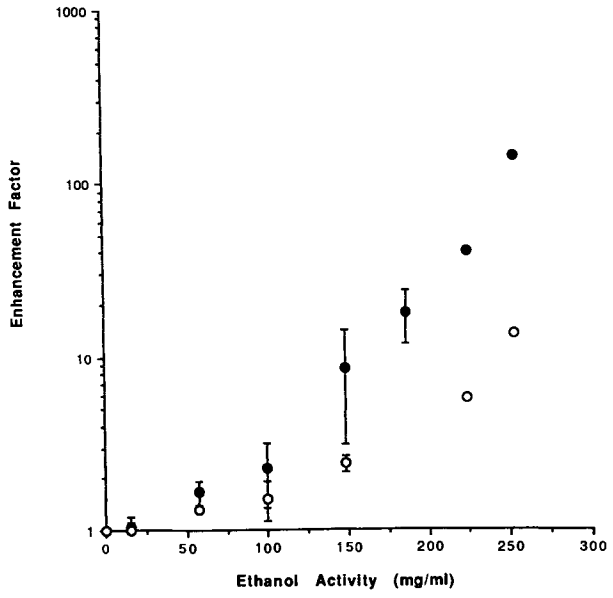

Fig. 4. "Symmetric" enhancement factors (E) of estradiol (filled circles) and of ethanol itself (open circles) in human epidermal membrane as a function of ethanol activity.

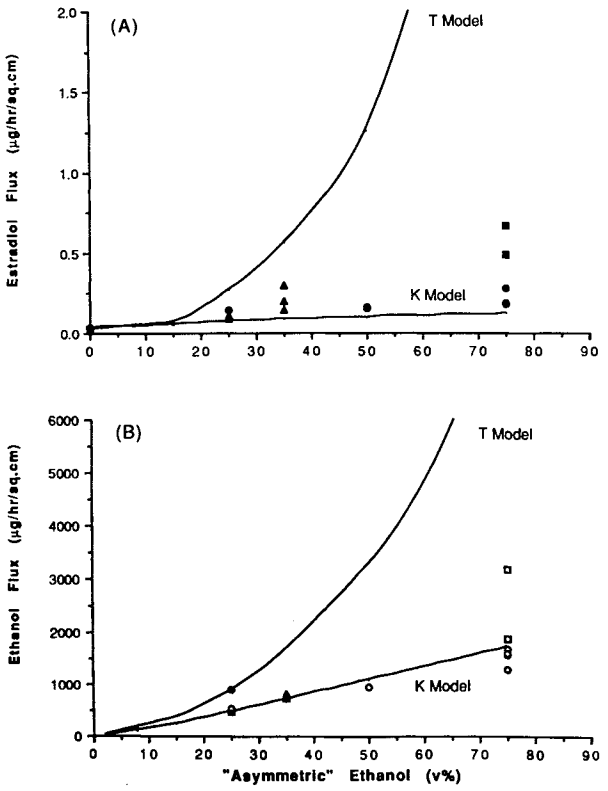

Fig. 5. Cotransported flux of saturated estradiol (A) and of ethanol (B) through human epidermal membrane as a function of ethanol concentration in the donor chamber ("asymmetric" configuration): comparisons of the experimental data (different symbols indicate different skin donors) and the model predictions (curves, average values) of the kinetic model (K model) and thermodynamic model (T model). Due to its dramatic increase (out of scale), T-model curves beyond 60% ethanol (A) and 65% ethanol (B) were not shown.

gion just beneath the polar head) is physically perturbed, such as an increase in structural disorder. Second, with increasing ethanol concentrations, in addition to the physical alteration, ethanol may partition into the lipoidal barrier region so that the chemical composition (polarity) of the barrier region is also changed. Finally, at higher ethanol levels (not involved in this study), a stronger physical/chemical interaction may cause secondary conformational changes within the keratinized protein component and partial extraction of the lipid barrier, resulting in formation of a new polar pathway. Biophysical techniques may provide further evidence in the confirmation of this hypothesis. In this study, the asymmetric fluxes for both saturated estradiol and ethanol vary with ethanol concentration (i.e., up to 75% in this study) in a manner consistent with a model which assumes that the ethanol gradient in the stratum corneum influences the transport of estradiol and ethanol itself by primarily increasing their diffusion coefficients at lower concentrations (<50%) and by both increasing their diffusion coefficients and decreasing their membrane activity coefficients at moderate concentrations (50 to 75%), where a position (x)-dependent kinetic/thermodynamic mechanism may be involved.

Permeant/Enhancer Flux Ratio: Model Predictions and Simulations

The permeant/enhancer flux relationship may be predicted from the permeant/enhancer enhancement factor relationship with the kinetic model [Eq. (K3)] and the thermodynamic model [Eq. (T3)]. Figure 6 shows the experimental estradiol/ethanol flux relationship compared with predictions of these two models. Both models show a nonlinear relationship between the permeant and the enhancer fluxes.

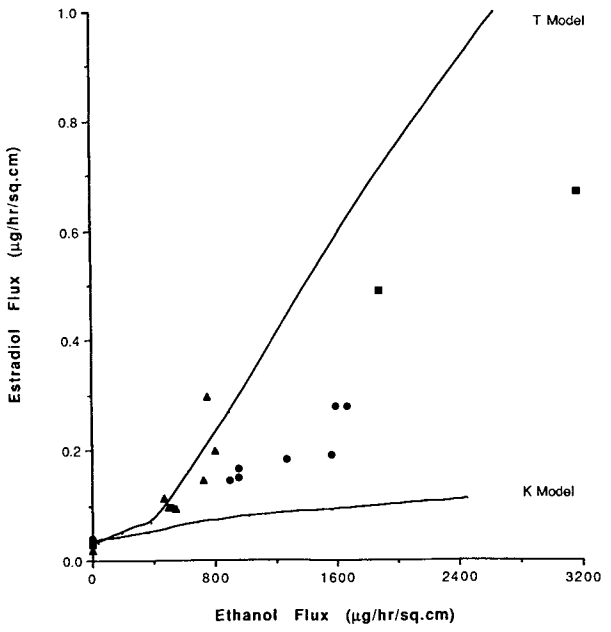

Fig. 6. Cotransported estradiol/ethanol flux relationship ("asymmetric" configuration): comparisons of the experimental data (different symbols indicate different skin donors) and the model predictions (curves, average values) of the kinetic model (K Model) and thermodynamic model (T model).

With its prediction uncertainty, the estradiol/ethanol flux relationship follows the kinetic model at lower ethanol flux. With increasing ethanol flux, the two models serve as the two limits of the experimental results.

In order to understand better the permeant/enhancer flux relationship in general, model simulations are conducted here for the permeant/enhancer enhancement factor ratio. Three limiting cases are well described in Table I. Case 1 is the simplified general model [Eq. (G3)], where both enhancer and permeant are enhanced by the same magnitude (i.e., $E_E = E_p > 1$). Cases 2 and 3 describe the situation where a large difference in the physicochemical properties of enhancer and permeant results in the enhancement of only one of them: case 2 represents enhanced permeant ($E_E \approx 1$ and $E_p > 1$) and case 3 represents self-enhanced enhancer ($E_p \approx 1$ and $E_E > 1$). From the information obtained from the estradiol/ethanol system, the exponentially increased enhancement factors (E_E and E_p) with enhancer activity are assumed in cases 2 and 3 for both the kinetic [Eq. (K3)] and the thermodynamic [Eq. (T3)] models.

Figure 7 summarizes the model simulations for five situations. All show nonlinearity of permeant/enhancer flux relationship for the full range of enhancer concentrations. The permeant/enhancer flux ratio becomes infinite as the permeant concentration (activity) approaches zero for all five model simulations. In cases 1 and 3, the permeant/enhancer ratio decreases in a parabolic manner with increasing enhancer concentration in the donor solution. The permeant/enhancer flux ratios, calculated from the thermodynamic

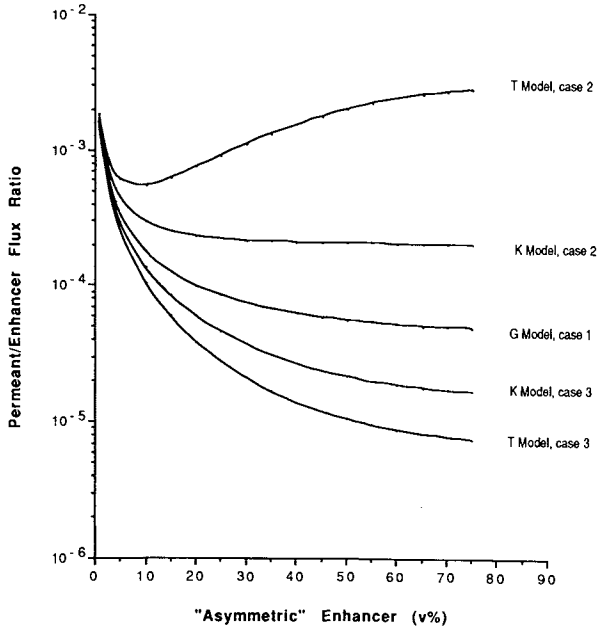

Fig. 7. Model simulations of permeant/enhancer flux relationships with five situations. (1) general model, case 1: Eq. (G3). (2) Kinetic model, case 2: Eq. (K3) with $E_E = 1$ and $E_p > 1$, and $E_p = \text{EXP}(\alpha_p a_E)$ where $\alpha_p = 16$ ml/g. (3) Thermodynamic model, case 2: Eq. (T3) with $E_E = 1$ and $E_p > 1$, and $E_p = \text{EXP}(\alpha_p a_E)$ where $\alpha_p = 16$ ml/g. (4) Kinetic model, case 3: Eq. (K3) with $E_p = 1$ and $E_E > 1$, and $E_E = \text{EXP}(\alpha_E a_E)$ where $\alpha_E = 7.5$ ml/g. (5) Thermodynamic model, case 3: Eq. (T3) with $E_p = 1$ and $E_E > 1$, and $E_E = \text{EXP}(\alpha_E a_E)$ where $\alpha_E = 7.5$ ml/g.

model, are more sensitive to changes of the enhancer concentration. Case 2 with the kinetic model gives a situation where the near-constant flux ratio starts at a relatively lower enhancer concentration (from around 30%) and approaches a constant (up to 75%). By examining Eqs. (K3), (T3), and (G3), one may deduce two conditions for an approximate linear permeant/enhancer flux relationship. The first one, for all five situations, is that the enhancer activity in the donor solution is less dependent on the enhancer concentration only at the higher ethanol concentrations. Another condition is for case 2 with the kinetic model [Eq. (K3)], where only the permeant is enhanced exponentially with enhancer activity so that the permeant/enhancer flux ratio approaches a constant at moderate enhancer concentrations in the donor solution.

In conclusion, the thermodynamic and kinetic limits of ethanol-enhanced estradiol skin transport have been investigated by studying the estradiol/ethanol flux relationship in the cotransport of estradiol and ethanol. A "symmetric"/"asymmetric" physical model approach has been used to determine the physical basis of the empirically linear permeant/enhancer flux correlation. The enhancement factors, determined with the symmetric configuration (the uniform ethanol concentration across stratum corneum), are used to predict fluxes in the asymmetric configuration (the ethanol gradient across stratum corneum). The asymmetric fluxes for both saturated estradiol and ethanol vary with ethanol donor concentration (i.e., up to 75% in this study) in a manner consistent with a model which assumes that the ethanol gradient influences the transport of estradiol and ethanol itself by increasing their diffusion coefficients at lower concentration (<50%) and by both increasing their diffusion coefficients and decreasing their membrane activity coefficients at moderate concentrations (50 to 75%). The model also demonstrates that the permeant flux is not simply linear with the cotransported enhancer flux in general. Moreover, when the two-parallel pathway enhancement occurs, separation of the enhancement contributions from each pathway must be considered for both enhancer and permeant. This approach may be applicable to other drug/vehicle cases where there is cotransport of a solvent that may act as a stratum corneum permeation enhancer.

APPENDIX

At steady state, in which the fluxes ($J_E^{*/o}$ and $J_p^{*/o}$) are independent of x, Eq. (5) may be integrated over either the full concentration gradient ($C_m = C_{m,d}^{*/o} \rightarrow 0$) or the whole membrane ($x = o \rightarrow h$), to yield

$$J^{*/o} = \frac{\int_0^{C_{m,d}^{*/o}} D^{*/o}\, dC_m}{h} \tag{A1}$$

$$J^{*/o} = \frac{C_{m,d}^{*/o}}{\int_0^h \frac{dx}{D^{*/o}}} \tag{A2}$$

Rearranging Eq. (A1) with Eq. (2), for enhancer, leads to Eq. (A3).

$$J_E^{*/o} = J_E^o \frac{\int_0^{C_{mE,d}^{*/o}} D_E^{*/o}\, dC_{mE}}{D_E^o C_{mE,d}^o} \tag{A3}$$

For permeant, noting that integration with position (x) is identical to integration with respect to enhancer concentration within the membrane (C_{mE}), one may transform Eq. (A2) into

$$J_P^{*/o} = \frac{C_{mP,d}^{*/o}}{\int_{C_{mE,d}^{*/o}}^0 \left(\frac{dx}{dC_{mE}}\right) \frac{dC_{mE}}{D_P^{*/o}}} \tag{A4}$$

Substituting the enhancer concentration gradient [Eq. (5)] into Eq. (A4), comparing it with the intrinsic flux [Eq. (2)], and combining it with Eq. (A3), one obtains the "asymmetric" flux for permeant,

$$J_P^{*/o} = J_P^o \frac{\gamma_{mP}^o}{\gamma_{mP,d}^{*/o}} \frac{\int_0^{C_{mE,d}^{*/o}} D_E^{*/o}\, dC_{mE}}{D_P^o \int_0^{C_{mE,d}^{*/o}} \frac{D_E^{*/o}}{D_P^{*/o}}\, dC_{mE}} \tag{A5}$$

The asymmetric permeant/enhancer flux ratios are obtained by combining Eqs. (A3) and (A5) and setting $\gamma^o{}_{v,E} = 1$.

$$\frac{J_P^{*/o}}{J_E^{*/o}} = \frac{J_P^o}{P_E^o} \frac{\frac{D_E^o}{D_P^o} \frac{\gamma_{mP}^o}{\gamma_{mE}^o}}{\gamma_{mP,d}^{*/o} \int_0^{C_{mE,d}^{*/o}} \frac{D_E^{*/o}}{D_P^{*/o}}\, dC_{mE}} \tag{A6}$$

For the kinetic model ($E = D^*/D^o$ with constant γ_m), the thermodynamic model ($E = \gamma_m{}^o/\gamma_m{}^*$ with constant D), and the simple general model (γ_{mP} and D_p are proportional to γ_{mE} and D_E, respectively, at any point within the membrane), Eqs. (A3), (A5), and (A6) may be simplified for the permeant and enhancer fluxes and the permeant/enhancer flux ratio (Table I).

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