# Analysis of Simultaneous Transport and Metabolism of Ethyl Nicotinate in Hairless Rat Skin

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***Purpose.*** Simultaneous skin transport and metabolism of ethyl nicotinate (EN), a model drug, were measured and theoretically analyzed.
***Methods.*** Several permeation studies of EN or its metabolite nicotinic acid (NA) were done on full-thickness skin or stripped skin with and without an esterase inhibitor. Permeation parameters such as partition coefficient of EN from the donor solution to the stratum corneum and diffusion coefficients of EN and NA in the stratum corneum and the viable epidermis and dermis were determined by these studies. Enzymatic parameters (Michaelis constant $K_m$ and maximum metabolism rate $V_{max}$) were obtained from the production rate of NA from different concentrations of EN in the skin homogenate. Obtained permeation data were then analyzed by numerical method based on differential equations showing Fick's second law of diffusion in the stratum corneum and the law with Michaelis-Menten metabolism in the viable epidermis and dermis.
***Results.*** Fairly good steady-state fluxes of EN and NA through the skin were obtained after a short lag time for all the concentrations of EN applied. These steady-state fluxes were not proportional to the initial donor concentration of EN: EN and NA curves were concave and convex, respectively, which suggests that metabolic saturation from EN to NA takes place in the viable skin at higher EN application. The steady-state fluxes of EN and NA calculated by the differential equations with resulting permeation and enzymatic parameters were very close to the obtained data.
***Conclusions.*** The present method is a useful tool to analyze simultaneous transport and metabolism of many drugs and prodrugs, especially those showing Michaelis-Menten type-metabolic saturation in skin.

**KEY WORDS:** skin permeation; skin metabolism; metabolic saturation; ethyl nicotinate; nicotinic acid.

## INTRODUCTION

Skin metabolism of topically applied drugs has been broadly investigated (1,2). These studies are important in detailed evaluations of the usefulness and safety of transdermal drug delivery systems. Several prodrug approaches are reported to be means of increasing skin permeation of a parent drug (3,4). A prodrug may be metabolized into a correspondent active parent drug in skin during the membrane transport. Recently peptides have also become candidates for transdermal delivery because they can be delivered through skin by physical penetration-enhancing methods like iontophoresis (5), phonophoresis (6), electroporation (7) and jet injection (8). The enzymatic barrier of the skin deserves more attention than the diffusion barrier as it is another important hurdle for the transdermal delivery of such a prodrug and peptide. Although a theoretical approach to the drug metabolism in skin can be beneficial in selecting a good prodrug or peptide candidate, it is somewhat difficult because diffusion and metabolism usually take place simultaneously in the viable skin (9). Saturation of the metabolic profiles makes a theoretical approach more difficult. Efforts toward a physical and mathematical approach of simultaneous transport and metabolism in skin were made (10); however, little experimental data were presented on the metabolic saturation during prodrug or peptide transport through skin.

In the present study ethyl nicotinate (EN) was selected as a model drug or prodrug, because the metabolism from EN to nicotinic acid (NA) is saturated in viable skin when EN is applied topically at high concentration. The aim of this study was to analyze the simultaneous transport and metabolism of EN in skin. The *in vitro* permeation of EN through excised hairless rat skin was first measured using different concentrations of its aqueous solution to obtain experimental data on its metabolic saturation in skin during the membrane permeation of the drug; the permeation and enzymatic parameters of EN were then measured in separate experiments; and finally, simultaneous transport and metabolism profiles of EN were simulated by the calculated parameters using differential equations showing Fick's second law of diffusion and the Michaelis-Menten metabolism process. The resulting simulated profiles were then compared with the experimental ones, the relation between the theoretical profiles and observed data was discussed, and usefulness of this theoretical approach was evaluated.

## THEORETICAL

### Physical Model for Simultaneous Transport and Metabolism of EN in Skin

EN is hydrolyzed by general esterases in skin. According to Potts et al. (11), it is assumed that the esterases are not in the stratum corneum (s.c.) but in the viable epidermis and dermis (v.e.d.). Figure 1 shows a schematic concentration-dis-

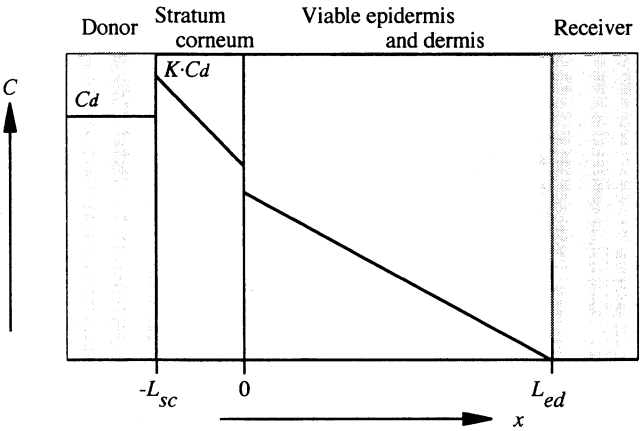


**Fig. 1.** Concentration and distance profile of ethyl nicotinate and nicotinic acid in skin after application of ethyl nicotinate (without diisopropyl fluorophosphate).

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tance profile of EN and NA from the donor to receiver compartment through skin. Two layers, s.c. and v.e.d. are estimated in skin in view of asymmetric enzyme distribution and different diffusivities of penetrants in the layers. Thickness of the two layers is set as $L_{sc}$ and $L_{ed}$, respectively. In this model, it is also assumed that NA hydrolyzed from EN in v.e.d. is not able to leach to the donor compartment through s.c. due to its higher s.c. permeability than EN (see results section for detail). The s.c. and v.e.d. are assumed to be homogeneous for diffusivities of EN and NA. Even esterase distribution in v.e.d. is also supposed in this model. Under these assumptions, we may describe simultaneous transport and metabolism of EN by Fick's second law of diffusion with having a non-linear reaction rate such as the Michaelis-Menten equation.

EN concentration in s.c., $C_{sc}$ at a position of $x$ and time $t$ can be represented as follows:

$$\frac{\partial C_{SC}}{\partial t} = D_{SC}\frac{\partial^2 C_{SC}}{\partial x^2} \tag{1}$$

where $D_{sc}$ is diffusion coefficient of EN in s.c. EN and NA concentrations in v.e.d., $C_{EN}$ and $C_{NA}$ are represented as follows (12):

$$\frac{\partial C_{EN}}{\partial t} = D_{EN}\frac{\partial^2 C_{EN}}{\partial x^2} + \frac{V_{\max}\cdot C_{EN}}{K_m + C_{EN}} \tag{2}$$

$$\frac{\partial C_{NA}}{\partial t} = D_{NA}\frac{\partial^2 C_{NA}}{\partial x^2} - \frac{V_{\max}\cdot C_{EN}}{K_m + C_{EN}} \tag{3}$$

where $D_{EN}$ and $D_{NA}$ are diffusion coefficients of EN and NA, respectively, in v.e.d., and $V_{max}$ and $K_m$ are the maximum metabolism rate and Michaelis constant.

Initial and boundary conditions of the penetrant concentrations in skin were set as:

$t = 0$

$-L_{SC} < x < 0 \quad C_{SC} = 0$

$0 \le x < L_{ed} \quad C_{EN} = C_{NA} = 0$

$t > 0$

$x = -L_{SC} \quad C_{SC} = KC_d$

$x = 0 \quad KC_{EN} = C_{SC}$ and $D_{SC}\frac{dC_{SC}}{dx} = D_{EN}\frac{dC_{EN}}{dx}$

$$\frac{dC_{NA}}{dx} = 0$$

$x = L_{ed} \quad C_{EN} = C_{NA} = 0$

where $K$ and $C_d$ are partition coefficient (s.c./vehicle) and donor concentration of EN, respectively. Time courses of $C_{sc}$, $C_{EN}$ and $C_{NA}$ at each position of skin, $x$, can be determined by eqns. 1–3 with these initial and boundary conditions. Fluxes of these compounds, $J_{EN}$ and $J_{NA}$ into the receiver compartment through the skin also can be obtained by the resulting $C_{EN}$ and $C_{NA}$ as follows:

$$J_{EN} = -D_{EN}\left(\frac{dC_{EN}}{dx}\right)_{x=L_{ed}} \tag{4}$$

$$J_{NA} = -D_{NA}\left(\frac{dC_{NA}}{dx}\right)_{x=L_{ed}} \tag{5}$$

Laplace transformation was used in order to reduce partial differential equations like eqns. 1–3 to an ordinary differential equation and finally to obtain a mathematical solution. In most cases, this technique enables us to elucidate solutions for drug concentrations at any time $t$ and any skin position $x$ as well as for drug fluxes through skin. In our cases, however, this technique cannot be applied because there is a non-linear reaction term, i.e., the Michaelis-Menten equation in eqns. 2 and 3. A numerical method using finite differences (12) was then applied. Differential terms in Fick's second law are approximated as follows:

$$\frac{dC_{i,j}}{dt} = \frac{1}{\Delta t}(C_{i,j+1} - C_{i,j}) \tag{6}$$

$$\frac{d^2C_{i,j}}{dx^2} = \frac{1}{\Delta x^2}(C_{i-1,j} - 2C_{i,j} + C_{i+1,j}) \tag{7}$$

where $C_{i,j}$ is penetrant concentration in the $i$-th layer at $j$-th time, and $\Delta x$ and $\Delta t$ are $x_{i+1} - x_i$ and $t_{j+1} - t_j$, respectively. By applying these approximations to eqns. 1–3,

$$C_{SC_{i,j+1}} = rD_{SC}C_{SC_{i-1,j}} + (1 - 2rD_{SC})C_{SC_{i,j}} + rD_{SC}C_{SC_{i+1,j}}$$

$$C_{EN_{i,j+1}} = rD_{EN}C_{EN_{i-1,j}} + (1 - 2rD_{EN})C_{EN_{i,j}} + \mathrm{r}D_{EN}C_{EN_{i+1,j}} - \Delta t\cdot\frac{V_{max}C_{EN_{i,j}}}{K_m + C_{EN_{i,j}}}$$

$$C_{NA_{i,j+1}} = rD_{NA}C_{NA_{i-1,j}} + (1 - 2rD_{NA})C_{NA_{i,j}} + rD_{NA}C_{NA_{i+1,j}} + \Delta t\cdot\frac{V_{max}C_{EN_{i,j}}}{K_m + C_{EN_{i,j}}}$$

where $r = \Delta t/\Delta x^2$. EN and NA fluxes at the dermis and receiver interface are then given by:

$$J_{EN} = -D_{EN}\frac{C_{EN_{n,j}} - C_{EN_{n-1,j}}}{\Delta x}$$

$$J_{NA} = -D_{NA}\frac{C_{NA_{n,j}} - C_{NA_{n-1,j}}}{\Delta x}$$

where $n$ is the last $i$, i.e., v.e.d. with a thickness of $L_{ed}$ is equally divided by $n$.

## Physical Model for EN and NA Transports Across Skin Pretreated with an Esterase Inhibitor

Figure 2 shows a schematic concentration-distance profile of EN or NA in skin. In this model it is also assumed that no hydrolysis from EN to NA takes place in v.e.d. or in s.c. due to the presence of an esterase inhibitor. Both layers are supposed to be homogeneous for diffusivities of EN and NA as true in the case in Fig. 1. Under these assumptions, EN and NA concentrations in s.c. and v.e.d., $C_{sc}'$ and $C_{ed}'$ at a position of $x$ and a time $t$ can be represented as follows:

$$\frac{\partial C'_{SC}}{\partial t} = D'_{SC}\frac{\partial^2 C'_{SC}}{\partial x^2} \tag{8}$$

$$\frac{\partial C'_{ed}}{\partial t} = D'_{ed}\frac{\partial^2 C'_{ed}}{\partial x^2} \tag{9}$$

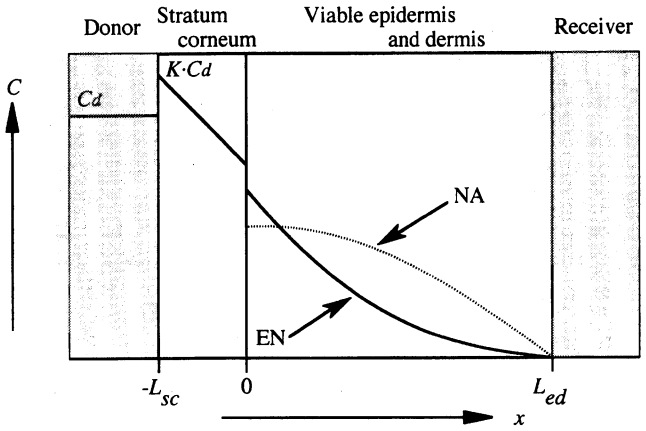


**Fig. 2.** Concentration and distance profile of ethyl nicotinate and nicotinic acid in skin after application of ethyl nicotinate or nicotinic acid with diisopropyl fluorophosphate.

where $D'_{sc}$ and $D'_{ed}$ are diffusion coefficient of EN or NA in s.c. and v.e.d. Initial and boundary conditions are:

$t = 0$

$-L_{SC} < x < 0 \quad C'_{SC} = 0$

$0 < x < L_{ed} \quad C'_{ed} = 0$

$t > 0$

$x = -L_{SC} \quad C'_{SC} = KC_d$

$x = 0 \quad KC'_{ed} = C'_{SC}$ and $D'_{SC}\frac{dC'_{SC}}{dx} = D'_{ed}\frac{dC'_{ed}}{dx}$

$x = L_{ed} \quad C'_{SC} = C'_{ed} = 0$

The amount of EN or NA permeated through skin into the receiver compartment, $\overline{Q}$ in the Laplace dimension can be solved by eqns. 8 and 9 and the initial and boundary conditions as above, according to Laplace transformation method.

$$\overline{Q} = \frac{KC_d}{sq_{ed}\{K\sinh(q_{ed}L_{ed})\cosh(-q_{SC}L_{SC}) - q_{SC}/q_{ed}\cdot\sinh(-q_{SC}L_{SC})\cosh(q_{ed}L_{ed})\}} \quad (10)$$

where $q_{sc} = \sqrt{s/D_{sc}}$ and $q_{ed} = \sqrt{s/D_{ed}}$.

In case of stripped skin, the final equation corresponding to eqn. 10 becomes,

$$\overline{Q} = \frac{KC_d}{sq_{ed}\sinh(q_{ed}L_{ed})} \quad (11)$$

### Estimation of Enzymatic Parameters, $K_m$ and $V_{max}$

The Hanes-Woolf plot (13) was then used to calculate enzymatic parameters, $K_m$ and $V_{max}$ on the hydrolysis rate from EN to NA in skin homogenates,

$$S/V = (1/V_{max})S + K_m/V_{max} \quad (5)$$

where $S$ and $V$ are substrate concentration and enzymatic reaction rate. In the Hanes-Woolf plot *(S/V vs. S)*, the slope is $1/V_{max}$ and the intercept of the ordinate gives the value of $K_m/V_{max}$. These parameters were obtained from enzymatic reaction rates of different concentrations of EN in skin homogenate using this equation.

## MATERIALS AND METHODS

### Materials

EN and NA were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) and diisopropyl fluorophosphate (DFP), an esterase inhibitor (14), from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Other chemicals and solvents were of reagent grade and used without further purification.

### Animals and Skin Membrane Preparations

Male hairless rats (WBN/IL-Ht) 9 weeks old, weighing about 200 g, were supplied by Life Science Research Center, Josai University (Sakado, Saitama, Japan) and were used in all animal experiments. The abdominal region of hairless rat skin was carefully shaved and excised before experiments. Stripped skin was obtained by 20 times of tape stripping s.c. from the shaved abdominal skin with adhesive cellophane tape (24 mm width, Nichiban Co., Tokyo).

### Stability Experiments Using Skin Homogenate

Freshly excised skin was homogenized using a nineteen-fold volume of 1/30 M phosphate buffered saline (pH 7.4) in an ice bath and the supernates were obtained by centrifugation at 9000 × g. Enzymatic hydrolysis rate of EN was determined in the 5% skin homogenate at 37°C. Enzymatic parameters were calculated as in the theoretical section.

Stability of EN in water and pH 7.4 phosphate buffered saline as well as that in skin homogenate was measured at 37°C. Three different concentrations (5, 10 and 20 mM) of EN were tested. The chemical degradation rate was much lower than the enzymatic hydrolysis rate (data not shown).

### *In Vitro* Membrane Permeation Procedure

A side-by-side diffusion cell set consisting of two half cells (15) was used to measure skin permeation of EN and metabolism from EN to NA as well as skin permeation of NA. Full-thickness skin or stripped skin was mounted between the two half cells, each 2.5 ml in volume and 0.95 $cm^2$ in effective diffusion area. The membrane was pretreated for 1 h by 2.5 ml of pH 7.4 phosphate buffered saline with and without 2.7 mM DFP in the dermis side and the same volume of water in s.c. side. Both sides were then replaced by 2.5 ml of phosphate buffered saline with and without DFP at a concentration of 0.54 mM and the same volume of EN solution or NA suspension, respectively, to start the permeation experiments. EN permeation alone (without metabolism from EN to NA) could be measured by the experiment with DFP. Concentration of EN was from 1.4 to 231.5 μmol/ml to expect linear to non-linear metabolism rate of EN in skin, and a saturated solution of NA (161.8 μmol/ml) was used. Both donor and receiver compartments were stirred with a star-head bar driven by a constant-speed synchronous motor (MC-301, Scinics, Tokyo) at about 1200 rpm. The permeation study was conducted at 37°C. The receiver solution was withdrawn every hour for up to 8 h and

analyzed for EN and NA by HPLC, and the same volume of fresh buffer was added to the cell to keep the volume constant.

### HPLC Analysis

EN and NA were determined by HPLC which consisted of a pump (LC-6A, Shimadzu, Kyoto, Japan), an UV detector (SPD-6A, Shimadzu), a 4.6 mm × 250 mm stainless-steel column packed with Nucleosil 5C18 (Macherey Nagel, Germany) and an integrator (C-R6A, Shimadzu). The mobile phase was 37.5: 62.5 of acetonitrile: pH 2.2 phosphoric acid solution containing 10 mM sodium l-hexanesulfonate, and the flow rate was 1.0 ml/min. Detection was by UV absorbance at 260 nm, and phenyl *n*-propylketone was used as an internal standard.

## RESULTS

Figure 3a and b shows typical *in vitro* profiles of simultaneous permeation and metabolism of EN without and with DFP, respectively, through excised hairless rat skin after applying aqueous solution of EN at a concentration of 145 μmol/ml in the donor compartment of a diffusion cell set. Without DFP, the cumulative amounts of EN and NA in the receiver solution increased linearly with time although a short lag time was found (Fig. 3a). Almost no efflux of NA was observed from the skin to the donor compartment during the 8 h-permeation experiments (data not shown). With DFP, little flux of NA was measured as shown in Fig. 3b.

The same kind of experiment was done using different concentrations of EN in the donor compartment. Figure 4a and b represents the steady-state flux of EN, NA and the sum of them through skin without and with DFP, respectively, against the initial donor concentration of EN. Interestingly, the total flux (sum of EN and NA flux) without DFP in Fig. 4a was very close to EN flux through the skin treated with DFP in Fig. 4b, which suggests that DFP has little effect on the diffusion of EN through skin. It was no wonder that EN flux with the enzyme inhibitor was proportional to the initial donor concentration of EN (Fig. 4b). It should be noted that the total flux was also proportional to the initial concentration (Fig. 4a). These permeation data with and without the esterase inhibitor suggest little contribution of metabolic barrier to the sum of the permeation rate of EN and NA.

These steady-state fluxes of EN and NA without DFP were not proportional to the initial donor concentration of EN applied on skin: EN and NA curves were concave and convex, respectively (Fig. 4a), which suggests that metabolic saturation from EN to NA takes place in skin at higher EN application.

The flux of EN and NA through stripped skin with and without the esterase inhibitor, respectively, was measured to determine permeation parameters such as partition coefficient of EN from water to s.c. and diffusion coefficients of EN and NA in s.c. and v.e.d. The partition coefficient was 32.1. Full-thickness skin permeability of NA was also determined without the esterase inhibitor. Diffusivity of NA through s.c. ($7.41 \times 10^{-8}$ cm$^2$/h) was about 30 times lower than that of EN ($2.05 \times 10^{-6}$ cm$^2$/h), so that NA leaching from the viable epidermis which was produced from EN was ignored. Diffusivity of EN in v.e.d. ($1.40 \times 10^{-2}$ cm$^2$/h) was closed to that of NA ($1.99 \times 10^{-2}$ cm$^2$/h).

Figure 5 shows a Hanes-Woolf plot to obtain the enzymatic parameters, $K_m$ and $V_{max}$ on the hydrolysis from EN to NA in the skin homogenate. $K_m$ and $V_{max}$ were calculated to be 0.837 μmol/ml and 5.70 μmol/cm$^2$/h, respectively, by the slope and intercept in the figure.

Solid lines in Fig. 4a show the calculated value by differential equations (eqns. 1–3) using the obtained permeation and enzymatic parameters. These lines were exactly the same as the observed values, which suggests that the present model for simultaneous transport and metabolism in skin is reasonable and that the obtained permeation and enzymatic parameters well reflect the real values in the skin during the permeation experiments.

## DISCUSSION

Simultaneous transport and metabolism after topical application of a drug are of interest to rationalize and develop a topical dosage form. Some investigators proposed a theoretical equation for the metabolic kinetics of a drug during its diffusion across skin (10,16). In a case having the first rate constant as

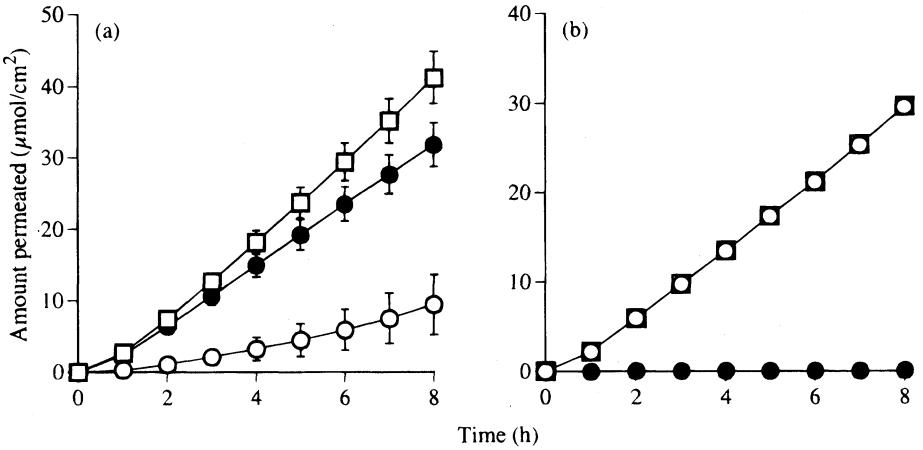


**Fig. 3.** Typical cumulative amount of ethyl nicotinate and nicotinic acid permeated through full-thickness skin without (a) and with diisopropyl fluorophosphate (b) Amount of application: 145 μmol/ml of EN solution (○): ethyl nicotinate, (●): nicotinic acid, (□): total Each point represents the mean ± S.E. of 5 experiments.

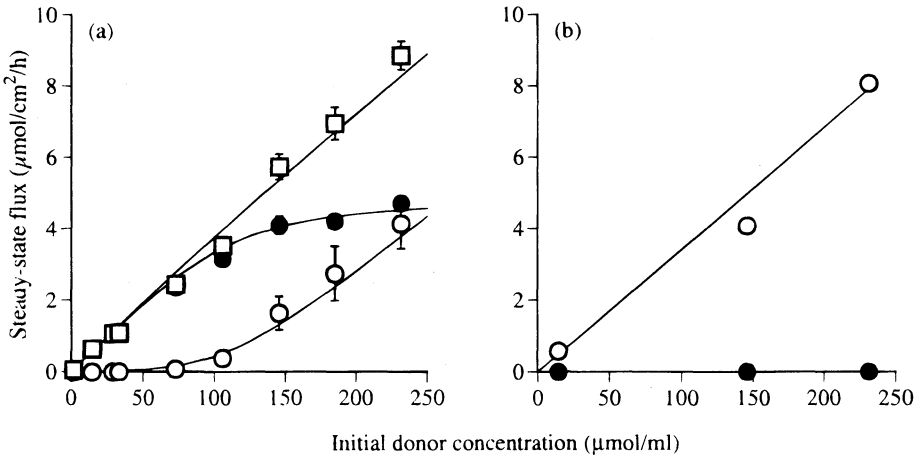


**Fig. 4.** Relations between fluxes of ethyl nicotinate and nicotinic acid through full-thickness skin without (a) and with diisopropyl fluorophosphate (b), and initial donor concentration of ethyl nicotinate (○): ethyl nicotinate, (●): nicotinic acid, (□): total Each point represents the mean ± S.E. of 5 experiments. Solid lines in (a) illustrate predicted value calculated by differential equations (eqns. 1–3) using obtained permeation and enzymatic parameters.

the metabolic process of a drug in skin to Fick's second law of diffusion, a Laplace transformed mathematical solution can be used to simulate drug disposition in skin after topical application and curve-fitting skin permeation data as well as blood level and urine excretion rate. The Michaelis-Menten equation was used as in the present paper to the Fick's equation by considering saturation of the drug metabolism in skin (12). No mathematical solution, however, was found for the diffusion equation with the Michaelis-Menten equation. Unfortunately, few observed data were reported for the metabolic saturation during drug permeation through skin, probably due to low skin permeation rate of a drug. Therefore, even if a mathematical method was offered, most reports described transport with the first ordered reaction (12). One objective of the present study was, thus, to present experimental data which show simultaneous transport and metabolic saturation of a drug in skin. EN was selected for this purpose. Theoretical analysis of these phenomena was also an important objective of this study.

First, enzymatic parameters are noted among the present data. The $V_{max}$ value for the metabolism of EN to NA obtained from an experiment using skin homogenate was 5.70 μmol/cm²/h, similar to the steady-state flux of NA at a plateau region as shown in Fig. 4a. In contrast, initial EN concentration in the donor compartment at a half of $V_{max}$ of the steady-state NA flux across skin (about 80 μmol/ml, Fig. 4a) was about 100 times higher than the $K_m$ value obtained from the experiment using skin homogenate (0.837 μmol/ml). The reason may be that the observed $K_m$ value corresponds to the EN concentration in skin, whereas the EN concentration as shown in the abscissa of Fig. 4a is its concentration in the donor compartment. However, EN concentration in skin during skin permeation is a function of not only time, $t$ but also distance from the skin surface, $x$. Even at the steady-state, therefore, it is difficult to determine true $K_m$ value from the flux data of NA in Fig. 4a.

Figure 6 shows a concentration-distance profile of EN and NA in skin at the steady-state after application of 100 μmol/ml EN on skin, which was calculated by a numerical method

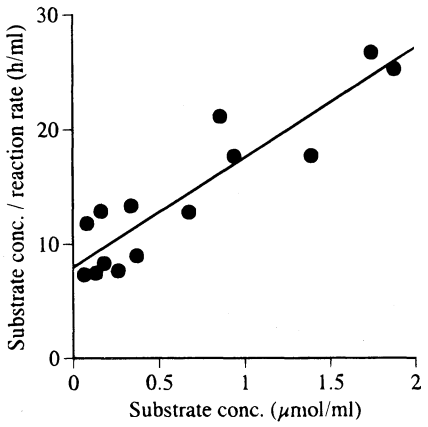


**Fig. 5.** Hanes-Woolf plot of ethyl nicotinate metabolism in skin homogenate supernates. Each point shows one data point.

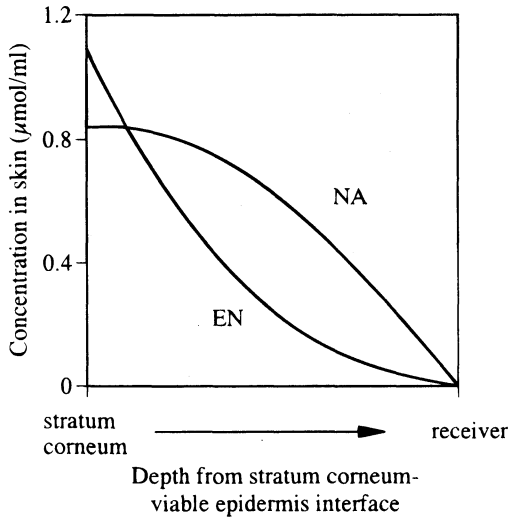


**Fig. 6.** Calculated steady-state concentration-distance profiles of EN and NA in viable epidermis and dermis.

using finite differences (see Theoretical section for detail). The profiles of EN and NA are concave and convex, respectively, which suggests that EN metabolism takes place everywhere in v.e.d.

Several problems remain in the present theoretical analysis on the simultaneous transport and metabolism of EN. We assumed an even esterase distribution in v.e.d. Tojo and his coworkers (10,16) suggested by an experiment using prednisolone esters that esterase distribution was not even and the esterase activity facing s.c. and at the end of dermis was higher than in the middle of v.e.d. in normal rats, and that totally opposite phenomena were found in the hairless mouse. Yu et al. (17) reported that deaminase activity in hairless mouse against 9-β-D-arabinofuranosyladenine was mainly in the shallow viable epidermis. Our preliminary experiment suggested that esterase activity in the middle of v.e.d. was higher compared to those in the shallower and deeper skin tissues (data not shown). Such localized enzyme distribution must have a marked effect on the EN and NA distribution in skin. Liu et al. (18) investigated epidermis-to-dermis transport and dermis-to-epidermis transport of β-estradiol. β-Estradiol is metabolized to estrone in viable skin: fluxes of both compounds were the same for both directions, whereas β-estradiol and esterone concentrations in skin were much different. Several simulations in the present study suggest that the distribution state of enzymatic activity is not too effective on the EN and NA fluxes across skin, but is on the EN and NA concentration-distance profiles in skin.

One other problem is a gap between the *in vitro* and *in vivo* skin permeations of EN. Average thickness of the present skin pieces from hairless rat was 850 μm. The systemic circulation system exists anatomically at 200–300 μm from the surface of the skin. EN and its metabolite NA may be uptaken mostly from the circulation system. Estimation of *in vivo* phenomena from the *in vitro* data is also an important subject in the simultaneous transport and metabolism of a drug in skin.

In conclusion, steady-state flux of EN and NA across skin after topical application of EN was simulated by differential equations of eqns. 1–3 using permeation parameters determined by the permeation experiment with DFP and enzymatic parameters like $K_m$ and $V_{max}$; the simulated lines were very close to the observed data points (Fig. 4a). The result suggests that the present model for simultaneous transport and metabolism of a drug across and in skin is reasonable and that the obtained permeation and enzymatic parameters well reflect the real values in the skin during the permeation experiments on a drug.

## ACKNOWLEDGMENTS

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