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Skin penetration of vitamins C and E

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Synopsis

The skin penetration profile of vitamin C determined by a HPLC assay procedure agreed approximately with that determined by liquid scintillation counting using the radiolabeled vitamin. This finding indicates that only a small amount of vitamin C is bioconverted to its metabolites in the hairless mouse skin. However, the lag time in the penetration profile assayed by HPLC was appreciably shorter than that determined by radioactivity counting. This finding suggests that the tissue vitamin C distributed initially either endogenously or exogenously might diffuse into the receptor solution during the transient period of penetration.

The penetration profile of vitamin E determined by HPLC differed markedly from that determined by radioactivity counting. During about 48 hours after the onset of the penetration experiment, the skin penetration of vitamin E remained almost negligible. Beyond that time, however, vitamin E appeared gradually in the receptor solution. By assuming first order kinetics of vitamin E bioconversion in the viable skin and the exponential decay law with respect to enzyme deactivation under *in vitro* conditions, the time course of the cumulative appearance of the vitamins after skin penetration was described on the basis of the bilayer diffusion/bioconversion model.

INTRODUCTION

During the last two decades, many researchers have revealed various biochemical functions of vitamins C (ascorbic acid) and E (α-tocopherol) (1,2,3). The use of these vitamins is unquestionably important not only for maintaining normal body metabolism but for preserving healthy skin. It was reported that vitamins C and E protect synergistically against the peroxidation of membrane lipid (4).

Various cosmetic formulations for skin care contain vitamins C or E as an active ingredient. However, the skin permeation of these vitamins has not been elucidated yet. We still lack much of the information we need to understand the percutaneous absorption of these vitamins.

In this communication, we have investigated the skin penetration of vitamins C and E using hairless mouse skin *in vitro*. The penetration profiles (time course of the cumulative amount of vitamin penetrated) for both radiolabeled and nonradiolabeled vitamins were determined using either HPLC or a liquid scintillation counter. The penetration

profiles were then analyzed by a dynamic mathematical model based on the bilayer skin diffusion/bioconversion model (5).

MATERIALS AND METHODS

Vitamin E (α-tocopherol) and vitamin C (ascorbic acid) were provided by Sigma Chemicals (St. Louis, MO). The radiolabeled vitamin E (($^3H)_2$-3,4α-tocopherol, 10–40 mCi/mmol) and vitamin C L-[1-^{14}C]-ascorbic acid, 10 mCi/mmol) were supplied by Hoffmann-La Roche (Nutley, NJ) and NEN Research (Massachusetts), respectively.

A full-thickness abdominal skin of a female hairless mouse (5–7 weeks old, Jackson Lab. HRS/J strain) was excised freshly before the *in vitro* skin penetration experiment. The skin sample was then mounted between the donor and receptor halfcells (Figure 1). The *in vitro* system, which has been calibrated with respect to hydrodynamic characteristics (6), assures the intrinsic skin penetration (7) under the present experimental condition. The donor and receptor solutions (Table I) were then charged in each cell compartment. At appropriate time intervals, 30 μl of the receptor solution was withdrawn and assayed for the vitamin concentration. Six sets of the *in vitro* diffusion cells were used in each penetration experiment. All experiments were carried out at a constant temperature (37°C).

The degradation of both vitamins in the donor solution was found to be negligible. In the receptor solution, however, the vitamins degraded appreciably during the penetration experiment. The penetration profile was therefore corrected for the degradation by

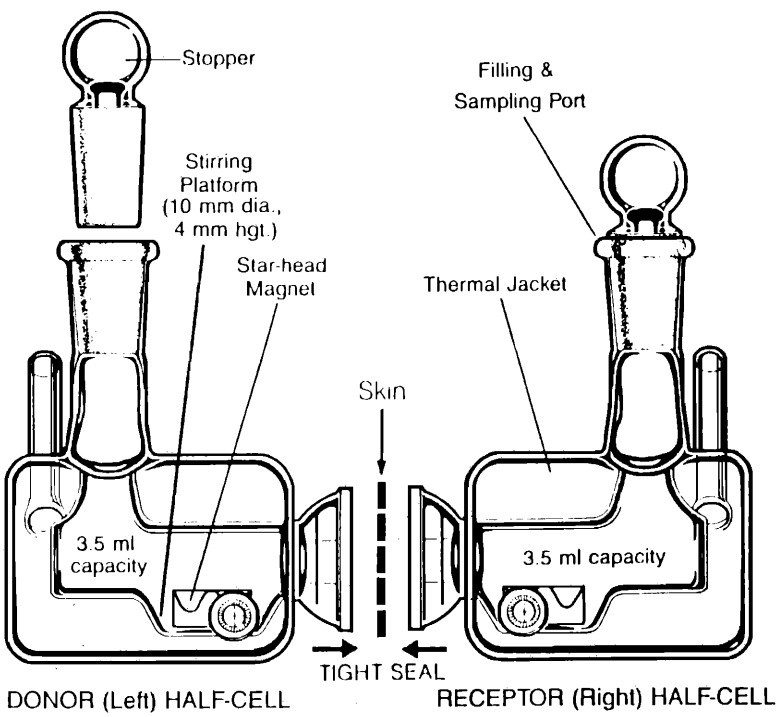

Figure 1. *In vitro* skin penetration apparatus used in this study.

Table I
The Donor and Receptor Solutions Used in the *In Vitro* Skin Penetration Study

Drug in donor	Donor solution	Receptor solution
Vitamin C	Non-radiolabeled vitamin C 12.11 ± 0.86 mg/ml and Radiolabeled ^{14}C-vitamin C 5.93 ± 0.25 × 10^5 DPM/ml in 50% glycerin aq. solution.	50% glycerin aq. solution
Vitamin E	Non-radiolabeled vitamin E 13.81 ± 0.51 mg/ml and Radiolabeled ^{3}H-vitamin E 1.23 ± 0.09 × 10^5 DPM/ml in silicone fluid (DC360, 20 cp)	5 mM Tween-80 aq. solution

the procedure described previously (8). The details of HPLC assay procedure for each vitamin were also reported previously (8).

The radioactivity of radiolabeled vitamins was measured by a liquid scintillation counter. The degradation of vitamins during skin penetration is not taken into account in this assay procedure. The radioactivity in the donor solution was 1.2 × 10^5 DPM/ml and 5.9 × 10^5 DPM/ml for vitamins E and C, respectively. During the entire period of penetration experiment, the receptor solution was maintained under a sink condition for both radiolabeled and nonlabeled vitamins.

The penetration profiles (time course of the cumulative amount of vitamin penetrated) were described by the bilayer diffusion/bioconversion model for percutaneous absorption (5) (Figure 2).

RESULTS AND DISCUSSION

VITAMIN C PENETRATION

The penetration profile (cumulative amount) of vitamin C determined by either HPLC or radioactivity counting is shown in Figure 3. After about nine hours, the penetration profile for both radiolabeled and nonlabeled vitamins reached a steady state. The rate of steady-state penetration under the HPLC assay condition evaluated from the slope of the linear portion of the profile was close to, but slightly lower than, that measured by radioactivity counting. This finding indicates that vitamin C was not metabolized in the skin to a significant degree. However, the time-lag, which is defined as the time intercept of the linear portion of the penetration profile, was appreciably shorter for HPLC assay (1.3 h) than that determined by radioactivity counting (3.9 h). We reported previously that vitamin C quickly appeared in the receptor solution after a provitamin bioconversion in the hairless mouse skin (8). The present finding, as well as our previous one, suggests that the initial tissue concentration of either endogenous or exogenous vitamin C may cause a bursting effect immediately after the onset of the penetration experiment. The tissue vitamin C in the hairless mouse skin (HRS/J strain) was recently demonstrated by Buettner *et al.* (9), although the concentration level has

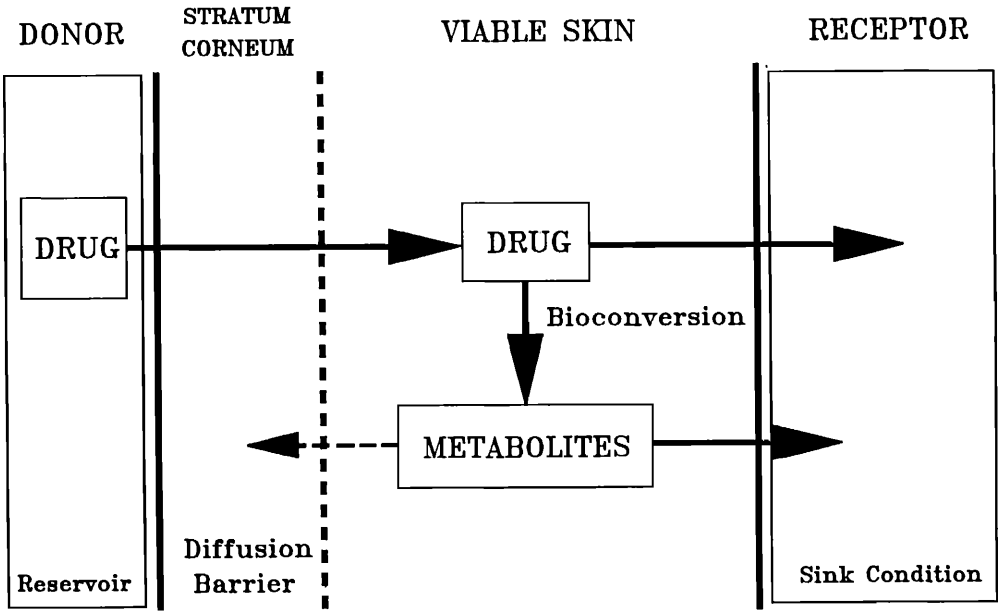

Figure 2. Bilayer diffusion/bioconversion model for percutaneous absorption. The drug concentration on the surface of the skin is assumed to be constant during the period of skin penetration (skin-controlled transdermal drug delivery system). The governing equations and boundary/initial conditions have been described in detail in reference (5).

not been elucidated. The penetration profile of vitamin C was theoretically analyzed by the dynamic bilayer-skin diffusion/bioconversion model (5). The diffusivity and the partition coefficient in each skin layer (stratum corneum and viable skin) were determined from the lag times and the steady-state fluxes on the basis of the steady-state bilayer skin model (10). By assuming a homogeneous distribution for tissue vitamin C (initial concentration), the penetration profile was simulated (Figure 3) and compared with the experimental one. The initial tissue concentration of vitamin C was found to be approximately 2.7 μmol/ml in the viable skin of the present animal model. The rate constant of vitamin C bioconversion in the skin was also found to be very small (1.5×10^{-5} s^{-1}) compared to the skin bioconversion of vitamin E discussed in the next section of this article.

VITAMIN E PENETRATION

The penetration profiles of vitamin E are plotted in Figure 4. The significant difference is easily observed in the penetration profiles between HPLC assay and radioactivity counting. The radiolabeled vitamin E penetrated promptly across the skin, while the nonlabeled compound appeared after a remarkably long lag time (about 48 hours). We found previously that vitamin E bioconverted in the viable skin from a provitamin appeared in the receptor compartment after a long lag time (24–36 hours) (8). We therefore suggested that vitamin E bioconverted in the viable skin might diffuse back into the stratum corneum very slowly. If this is the case, the penetration profile not only of the radiolabeled vitamin E but also of the nonlabeled compound should provide a long lag time. Contrary to our previous hypothesis, the significant difference appears in the penetration profiles between radiolabeled and nonlabeled compounds. The long

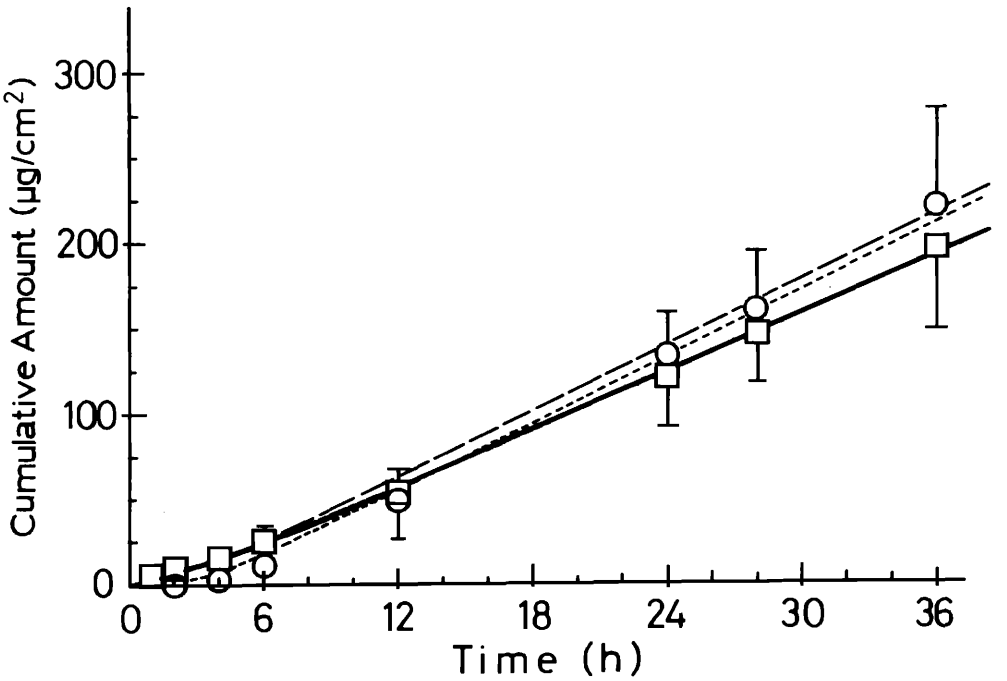

Figure 3. Penetration profile of vitamin C across hairless mouse skin. Key: (□) HPLC assay. (○) Radioactivity counting. ----- Calculated (initial concentration in viable skin C_0 = 2.7 μmol/ml, no bioconverson). ..... Calculated (C_0 = 0, no bioconversion). —— Calculated (initial concentration in viable skin C_0 = 2.7 μmol/ml, first order bioconversion kinetics $k_0 = 1.5 \times 10^{-5}\ s^{-1}$, enzyme decay rate constant $A = 5.6 \times 10^{-6}\ s^{-1}$). The diffusivity and the partition coefficient both in the stratum corneum and in the viable skin were determined from the penetration profiles of radiolabeled vitamins across the intact and stripped skins. The detailed procedure has been described in reference (10). Diffusivity in stratum corneum = 7.6×10^{-11} cm²/s. Diffusivity in viable skin = 5.8×10^{-8} cm²/s. Partition coefficient between stratum corneum and viable skin = 0.28. Concentration on the skin surface = 136 μmol/ml. Thickness of stratum corneum = 10 μm. Thickness of viable skin = 370 μm.

lag time in the penetration profile assayed by HPLC therefore suggests the extensive metabolism of vitamin E in the skin. At this stage of research on percutaneous absorption of vitamins, little is known with respect to the skin metabolism. However, Shiratori suggested that the skin may be an important storage site for vitamin E and play a major role in distribution and metabolism of vitamin E (11).

After about 48 hours, vitamin E appeared gradually in the receptor solution. This is due to the fact that skin enzyme becomes gradually deactivated under the *in vitro* condition. The enzyme which is responsible for esterification of estradiol esters was found to degrade in the hairless mouse skin under the *in vitro* condition by following the exponential decay law (12). Assuming the exponential decay law for the activity of skin enzymes,

$$k = k_0 \exp(-At) \tag{1}$$

where A is the decay rate constant, k_0 is the intrinsic rate constant for bioconversion, and t is the time, the experimental profiles of vitamin E penetration were described by the present model. The results are shown in Figure 4 where the calculated profile based on the constant activity of enzymes (A = 0) is also plotted for comparison. The calcu-

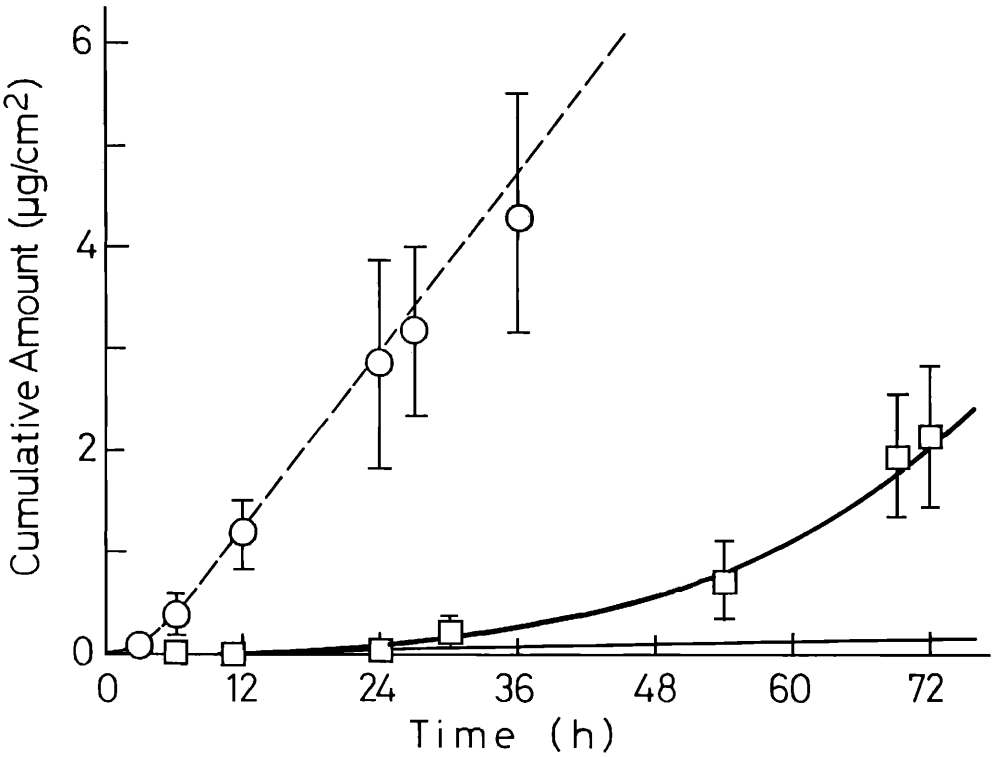

Figure 4. Penetration profile of vitamin E across hairless mouse skin. Key: (□) HPLC assay. (○) Radioactivity counting (× 1/10). ▬ Calculated (k_0 = 0.0038 s^{-1}, A = 5.6 × 10^{-6} s^{-1}). ___ Calculated (k_0 = 0.0038 s^{-1}, A = 0). ----- Calculated (× 1/10) (k_0 = 0, no metabolism). Diffusivity in stratum corneum = 8.4 × 10^{-11} cm^2/s. Diffusivity in viable skin = 7.1 × 10^{-8} cm^2/s. Partition coefficient between stratum corneum and viable skin = 18.9. Concentration of the skin surface = 22.3 μmol/ml. Thickness of stratum corneum = 10 μm. Thickness of viable skin = 370 μm. The numbers on the curves are the values of decay rate constant A.

lated profile with the rate constant A of 5.6 × 10^{-6} s^{-1} agrees fairly well with the experimental profile. It is also found that the enzyme activity in the hairless mouse skin, if freshly excised, remains unchanged during at least 24 hours after the onset of the *in vitro* skin penetration experiment.

CONCLUSION

The present study clearly indicates that the permeation of a radiolabeled compound appears to be significantly higher than the penetration of the actual compound due to skin bioconversion. It is therefore important, when evaluating percutaneous absorption of drugs, not only to determine the overall permeation rate but also to investigate whether or not enzymatic bioconversion occurs in the skin.

ACKNOWLEDGMENT

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