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Research Article

Percutaneous Absorption of Benzoic Acid Across Human Skin. I. *In Vitro* Experiments and Mathematical Modeling

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The percutaneous absorption of benzoic acid across human skin *in vitro* was experimentally and mathematically modeled. Skin partition coefficients were measured over a range of benzoic acid concentrations in both saline and distilled water. The permeation of benzoic acid was measured across isolated stratum corneum, stratum corneum and epidermis, and split-thickness skin. These experiments demonstrated that the stratum corneum was the rate-limiting barrier and that the flux is proportional to the concentration of the undissociated species. The permeation data were analyzed with a comprehensive non-steady-state mathematical model of diffusion across skin. Two adjustable parameters, the effective skin thickness and diffusivity, were fit to the permeation data by nonlinear regression.

KEY WORDS: percutaneous absorption; mathematical model; human skin; partition coefficient; *in vitro;* benzoic acid.

INTRODUCTION

The permeation of compounds across the skin is an important area of pharmaceutical and toxicological research. The skin acts as an efficient barrier to the permeation of molecules and protects the body from the absorption of foreign compounds and from the dehydration of body fluids. Notwithstanding the barrier property of skin, the topical application of drugs is an advantageous route of drug administration. The problem of slow percutaneous absorption has stimulated many investigations into the fundamentals of diffusion across skin.

The uppermost section of skin, the stratum corneum, consists of a 10- to 50-μm-thick layer of anucleated keratinized cells (1). This thin layer of cells frequently constitutes the primary resistance to the diffusion of compounds across skin. The underlying layer of skin, the epidermis, is composed of proliferating cells with a thickness of 50 to 100 μm and an effective diffusivity from one to three orders of magnitude greater than that found in the stratum corneum. The deepest layer of skin, the dermis, is a capillary-perfused region that operates as a sink for the uptake of most compounds that have permeated across the skin.

The flux of compounds is measured across skin with various *in vitro* and *in vivo* techniques. The objective of this study was to develop and validate theoretical descriptions and experimental *in vitro* protocols for predicting *in vivo* permeation of compounds. If *in vitro* measurements can be extrapolated reliably to describe the behavior of the *in vivo* system, costly and difficult *in vivo* experimentation can be avoided. In many studies, *in vitro* experiments attempt to simulate the *in vivo* situation, and the amount of *in vitro* drug absorption is compared directly to the amount of drug absorption measured in human subjects (2). Our strategy, similar to Scheuplein's (3), was to design *in vitro* experiments that obtain physical parameters such as diffusivity, partition coefficient, and skin thickness, which are then used to calculate *in vivo* permeation.

Benzoic acid has served as a model compound for numerous studies of skin permeation (4–8). In this paper, the parameters controlling permeation of benzoic acid were quantified with *in vitro* experiments using excised human skin. Benzoic acid partition coefficients between skin and vehicle were determined with equilibrium bottle tests. Percutaneous absorption of benzoic acid across skin was measured with side-by-side diffusion cells. A mathematical model of the permeation experiments was developed and used to fit values of effective diffusivity and skin thickness to the permeation data. These *in vitro* permeation parameters were used subsequently in a mathematical model to predict the *in vivo* percutaneous absorption of benzoic acid across a human-skin-sandwich-flap rat model (9).

THEORETICAL

The experimental protocol for permeation experiments, shown schematically in Fig. 1, involved placing a solution of a compound in the donor chamber and monitoring the accu-

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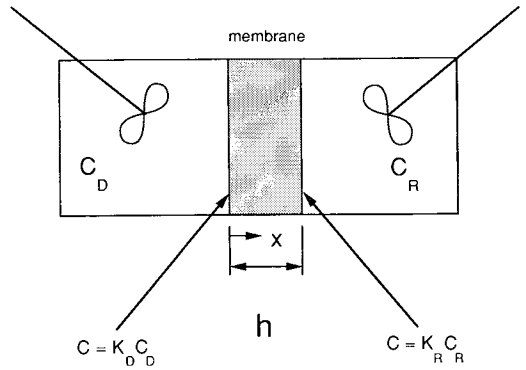

Fig. 1. Schematic diagram of *in vitro* permeation experiment.

mulation of that compound in the initially solute-free solution of the receiving chamber. This *in vitro* permeation system was described mathematically with a non-steady-state model summarized in Table I and Table II (all variables are defined under Nomenclature).

The key components of the model are as follows: (i) well-stirred donor and receiving compartments; (ii) depletion from the donor chamber; (iii) accumulation in the receiving chamber; (iv) instantaneous equilibrium at the skin–solution interfaces; (v) homogeneous stratum corneum as the principle barrier to diffusion; (vi) constant diffusion coefficient; and (vii) no binding or metabolism within the skin. The model assumes that the solution-to-skin equilibrium is represented by partition coefficients that depend on the vehicle which is in contact with the skin. Assumption iii allows for drug concentration buildup in the receiving chamber thereby removing the experimental constraint to maintain sink conditions.

The set of the differential equations and restricting conditions in Table I describe the changes of solute concentration in the skin, donor chamber, and receiving chamber as a function of time. The solution to this system of equations, obtained by Laplace transforms, is given in Table II.

Table I. Mathematical Description of Non-Steady-State *in Vitro* Permeation Experiment

Diffusion in the Skin		
$\frac{\partial C_m}{\partial t} = D_m \frac{\partial^2 C_m}{\partial X^2}$		
$C_m = 0$	@	$t = 0$
$C_m = K_D C_D$	@	$X = 0, t > 0$
$C_m = K_R C_R$	@	$X = h, t > 0$
Donor Chamber		**Receiving Chamber**
$V_D \frac{\partial C_D}{\partial t} = AD_m \frac{\partial C_m}{\partial X}\Big\vert_{X=0}$		$V_R \frac{\partial C_R}{\partial t} = -AD_m \frac{\partial C_m}{\partial X}\Big\vert_{X=h}$
$C_D = C_D^0$	@ $t = 0$	$C_R = 0$ @ $t = 0$
$C_m = K_D C_D$	@ $X = 0$	$C_m = K_R C_R$ @ $X = h$

If the concentrations of the receiving and donor chamber solutions change negligibly, the system of equations in Table I is solved with the result that, at long times, the accumulation of solute passing through an area of the membrane, M/A, is given as (10)

$$\frac{M}{A} = \frac{D_m K_m C_D}{h}\left[t - \frac{h^2}{6D_m}\right] \tag{1}$$

where K_m is the skin-to-solution partition coefficient and C_D is the concentration of drug in the donor chamber. The diffusion coefficient for benzoic acid in the skin D_m and the skin thickness h are effective parameters for a hypothetical homogeneous membrane exhibiting the same permeation behavior as the stratum corneum. That is, D_m essentially lumps the effects microscopic and molecular heterogeneities of the stratum corneum structure and composition into a single averaged value. Likewise, we would expect that the effective skin thickness h will be larger than the measured stratum corneum thickness, reflecting that diffusing molecules may follow a tortuous, intercellular lipid pathway (11). In addition, the chemical nature of the diffusing solute (perhaps lipophilicity or size) might influence the availability of certain path options, leading to effective path lengths which are different for different solutes.

Theoretically, one can estimate both h and D_m for a composite medium like skin. However, to do so requires information which is not yet known. A more complete description of the skin would require the quantities and geometric distribution of the phase regions (presumably intercellular lipids and intracellular keratin) and the molecular diffusivities and partition coefficients for the transferring solute in each phase regime. Given the current understanding of the stratum corneum structure and its relationship to local partitioning and molecular diffusion, explicitly treating its heterogeneous character in our transport modeling is not warranted.

The steady-state flux is found by differentiating Eq. (1) with respect to time,

$$\frac{dM/A}{dt} = \frac{D_m}{h} K_m C_D = P C_D \tag{2}$$

where the proportionality constant, P, describes the permeability of the skin to the compound of interest and collectively lumps the effects of phase partitioning, diffusivity, and skin thickness.

To be dimensionally correct, the partition coefficient K_m in Eqs. (1) and (2), when multiplied by the donor concentration C_D, given as solute mass per volume of solution, must yield the concentration of solute in the skin surface in units of solute mass per volume of hydrated skin. That is, K_m is defined as

$$K_m = \frac{\text{mass of drug/volume of hydrated skin}}{\text{mass of drug/volume of solution}} \tag{3}$$

Some researchers have reported partition coefficients K'_m calculated on the basis of dry skin mass and mass of solution defined as

Table II. Solution of Equations Shown in Table I

The accumulation of compound in the receiving chamber is defined by the following expression:

$$M = C_D^0 \frac{K_D}{K_R} V_R \theta_R$$

where

$$\theta_R = \left[1\Big/\left(\gamma_D + \frac{\gamma_D}{\gamma_R} + 1\right)\right] + \sum_{n=1}^{\infty} \{\gamma_R \exp[-\lambda_n^2 \tau]\Big/\left[\left[\frac{-3\lambda_n}{2} + \frac{\gamma_D\gamma_R}{2\lambda_n} - \frac{(\gamma_D + \gamma_R)\lambda_n}{2}\right] \sin \lambda_n + \left[\frac{-\lambda_n^2}{2} + \frac{\gamma_D\gamma_R}{2} + \gamma_D + \gamma_R\right] \cos \lambda_n\right]\}$$

λ_n are the roots of

$$\gamma_D + \gamma_R - \lambda_n \left[1 - \frac{\gamma_D\gamma_R}{\lambda_n^2}\right] \tan \lambda_n = 0$$

and the following symbols are defined as

$$\theta_R = \frac{K_R C_R}{K_D C_D^0}, \quad \tau = \frac{Dt}{h^2}, \quad \gamma_D = \frac{AK_D h}{V_D}, \quad \gamma_R = \frac{AK_R h}{V_R}$$

$$K'_m = \frac{\text{mass of drug/mass of dry skin}}{\text{mass of drug/mass of solution}} \tag{4}$$

The dimensionless quantities K_m and K'_m are related according to

$$K_m = K'_m \frac{\rho_{sc}}{\rho_D} \tag{5}$$

where ρ_D is the solution density and ρ_{sc} is not a true density but rather the ratio of the mass of dry skin to hydrated skin volume. The swollen, hydrated volume of the skin varies with the vehicle, but typical values for ρ_{sc} are 0.1 to 0.2 g/ml.

Knowing skin permeability P and an effective skin thickness h, one can calculate an apparent diffusion coefficient,

$$D_m = \frac{Ph}{K_m} \tag{6}$$

Importantly, effective diffusion coefficients incorrectly calculated using K'_m rather than K_m in Eq. (6) without accounting for the ratio ρ_{sc}/ρ_D will be low by as much as one order of magnitude while appearing to be dimensionally consistent.

As indicated by Eq. (1), at steady-state permeation, the mass accumulation of solute in the receiving chamber is a linear function of time with a slope equal to the skin permeability and a time axis intercept at a finite value, commonly called the lag time, which from Eq. (1), is

$$t_{lag} = \frac{h^2}{6D_m} \tag{7}$$

Barry (10) suggested calculating the diffusivity of a compound from the measured lag time and a measured or assumed skin thickness. The partition coefficient is then calculated from the permeability using Eq. (6) after rearrangement.

This approach has two problems. First, it is difficult to estimate or measure a skin thickness approximating the average diffusion path length which the permeating solute actually experiences. This uncertainty in h directly affects D_m and K_m, which are calculated using h. The second problem is that even small errors in values of receiving chamber mass accumulations at longer times can lead to large errors in the lag times computed from linear extrapolations of steady-state permeation data. As a result, the linear extrapolated time intercepts are occasionally negative or, at least, susceptible to large variability. In part, this situation arises because finding P and t_{lag} from the slope and intercept of a linear fit to the experimental data ignores that P and t_{lag} are both themselves functions of skin thickness and diffusivity, specifically in the form D_m/h and h^2/D_m, respectively. A better procedure is to seek values for D_m and h, rather than P and t_{lag}, which best fit the linear steady-state experimental results.

Our approach was independently to measure the partition coefficient and then calculate values for the effective thickness and diffusion coefficient which best fit the experimental permeation data to the complete non-steady-state mathematical model given in Table II. The maximum-likelihood estimation of D_m and h were calculated with a Levenberg–Marquardt nonlinear least-squares method (12), weighted by the standard deviation at each time point of the receiving chamber mass accumulation data from multiple experiments.

As already discussed, fitting on D_m and h rather than P and t_{lag} forces calculated values for D_m and h to be physically consistent. The non-steady-state model in Table II accounts for the unsteady flux at short times as well as receiving chamber accumulation or donor chamber depletion at longer times. The advantage of fitting the experimental data to the complete non-steady-state model is that the approach is general and short- and long-time data which might not be in the steady-state region can be used.

MATERIALS AND METHODS

Materials

A saturated solution of benzoic acid (Sigma Chemical Company) was kept in distilled water at 35°C (5 mg/ml benzoic acid at pH 2.75) and spiked with radiolabeled [^{14}C]-benzoic acid (New England Nuclear Corporation) to produce a specific activity of 3.0×10^{-5} mCi/mg. TLC analysis showed that no significant degradation of benzoic acid was apparent after experimentation (purity > 97%). Isopropyl myristate was obtained through Sigma Chemical Company. Saline solution, 0.9% sodium chloride irrigation, USP, was obtained from Abbott Laboratories.

Skin Samples

Abdominal female human skin was obtained from elective abdominoplasty surgery and received within 24 hr of surgery. The skin was dermatomed to a thickness of 500 μm and stored for up to 2 weeks in tissue culture medium (Dulbecca's modified Eagles medium with 5% fetal calf serum, Flow Laboratories, McLean, VA) at 4°C.

Isolated stratum corneum was obtained (13) by placing dermatomed skin, dermis side down, on a Whatman No. 1 filter paper soaked with 1% trypsin solution (No. T-1034 Type IX, Sigma, St. Louis, MO) adjusted to pH 8. After sitting for 2 hr at room temperature, the stratum corneum was separated from the epidermis and promptly placed in tissue culture medium to deactivate the trypsin. The skin was then rinsed in distilled water and the remaining epidermal material adhering to the stratum corneum was removed with a cotton-tipped applicator.

Stratum corneum with intact epidermis was obtained by placing dermatomed skin in water at 60°C and gently swirling the skin for 2 min. The skin was removed from the water and placed dermis side down on a paper towel where the stratum corneum–epidermis layer of skin was separated from the dermis. After separation by either technique, the skin was placed in distilled water at 4°C until used for experimentation. The water in which skin samples were stored was replaced by fresh water every 3 days. In all cases, the separated skin was stored less than 3 days before experimentation, except for the set of experiments designed to study the effect storage time has on changes in permeability.

Analytical Methods

The drug content in the various liquid samples collected from permeation and partition experiments was determined by scintillation counting. The liquid samples were added to 9 ml of Packard Opti-Fluor in 25-ml scintillation vials and analyzed for radioactivity with a Beckman LS 8100 liquid scintillation system. Radioactive drug content in skin was measured by placing the skin in a scintillation vial with 200 μl of Beckman tissue solubilizer 450 (BTS) and allowing the skin to digest for 2 hr at 40°C. Following digestion, 100 ml of 30% hydrogen peroxide was added to the vial and set at 40°C for 1 hr to decolorize the solution. Seven milliliters of scintillation cocktail solution was then added to the vial and the radioactivity was measured by scintillation counting.

Partition Coefficients

Partition coefficients were measured between distilled water and separated human skin. Circular pieces of skin were obtained from either isolated stratum corneum or heat-separated skin with a 1.4-cm-diameter cork bore. The volume of these skin pieces was calculated by determining the area and measuring the thickness of the skin with an engineer's micrometer (Mitutoyo No. 7326, Japan; sensitivity of 0.0001 in.). Skin samples were placed in 2-ml microcentrifuge tubes containing 2 ml of radiolabeled benzoic acid solution and incubated at 35°C in a water bath. Partition coefficients were measured in benzoic acid solutions with concentrations that ranged from 0.10 to 5.0 mg/ml.

Following equilibration for 24 hr, the skin was removed from the radioactive solution and blotted dry. The radioactivity in the skin and in a 200-μl sample of the final solution of benzoic acid was then measured. The skin concentration was calculated by dividing the amount of benzoic acid in the skin by the volume of the hydrated skin sample.

The partition coefficient of benzoic acid from distilled water to isopropyl myristate was measured by placing 2 ml of the immiscible liquids in a glass vial. Radiolabeled benzoic acid was added to the mixture, vortexed for 1 min, and equilibrated between the two phases for 48 hr at 35°C. The radioactivity in 200-μl samples of each phase was quantified with liquid scintillation counting.

Permeation Experiments

Permeation of benzoic acid in distilled water was measured across isolated stratum corneum, stratum corneum and epidermis (heat-separated skin), and split-thickness skin (dermatomed). Pieces of skin were clamped between the ground-glass flanges of two side-by-side diffusion chambers. The 3-ml chambers had a permeation area of 0.785 cm^2. The stratum corneum was set facing the donor chamber, which was filled with the supernatant from the benzoic acid saturated solution (5 mg/ml), and the receiving chamber was filled with distilled water containing no benzoic acid.

The permeation cells were placed in a circulating water bath at 35°C and the chambers were stirred by Teflon-coated magnetic stir bars at 200 rpm. Yu (14) previously reported a hydrodynamic boundary in similar cells stirred at 180 rpm. This thickness corresponds to a permeability of about 3.6 cm/hr assuming a benzoic acid diffusivity in water of 10^{-5} cm^2/sec. As shown later, the hydrodynamic boundary layer is responsible for less than 1% of the total measured mass transfer resistance. Sample volumes of 100 μl were periodically withdrawn from the receiving chamber and replaced with distilled water over a period of 4 hr. Samples were also collected from the donor chamber before and after the permeation experiment. Radioactivity within the skin was quantified at the completion of the permeation experiment.

RESULTS AND DISCUSSION

For solutes that ionize like benzoic acid, the ionized form of the compound is minimally soluble within the stratum corneum (1). As a result, the amount of benzoic acid partitioning into skin depends on the concentration of the undissociated species in solution. The degree of dissociation

is calculated knowing the amount of benzoic acid added, the acid dissociation constant, and the concentration of any other added bases or acids. The pK_a for benzoic acid at 35°C (4.04) was estimated from the pK_a reported at 25°C [4.19 (15)] adjusted using the van't Hoff equation (16) [ΔH dissociation = −6501 gcal/gmol (17)]. At 35°C, the total and undissociated benzoic acid concentrations in a saturated solution with no added base are 5.0 and 4.8 mg/ml, respectively.

The skin–vehicle partition coefficient was calculated as the slope of a regression fit forced through the origin (SPSS/PC, version 3.0, Microsoft Corp., 1988) where benzoic acid concentrations in the skin and vehicle (undissociated species only) were the dependent and independent variables, respectively. The partition coefficient of undissociated benzoic acid from excised human skin to distilled water was measured as 4.8 ± 0.2 (n = 29; six skin sources) for stratum corneum and 5.0 ± 0.3 (n = 30; six skin sources) for heat-separated skin (stratum corneum and epidermis). Figure 2 shows the benzoic acid concentrations in skin and vehicle, along with the regression fit for isolated stratum corneum. The similar partition coefficients for heat-separated skin and isolated stratum corneum indicate an equal affinity for benzoic acid by both the stratum corneum and the epidermis. The partition coefficient between isolated stratum corneum and saline was found to be 3.8 ± 0.1 (n = 15; two skin sources).

As an approximation to the skin–vehicle partition coefficient, many investigators replace the skin phase with isopropyl myristate. Liquid–liquid partition coefficients are easier to measure and might indicate skin–vehicle partitioning trends for compounds in question. The isopropyl myristate–water partition coefficient for undissociated benzoic acid was measured as 4.2, which is similar to the stratum corneum–water partition coefficient of 4.8.

For the permeation experiments, benzoic acid accumulation in the receiving chamber was measured as a function of time. The accumulations at each sample time were averaged over all experiments using isolated stratum corneum (n = 8; four skin sources) and are graphed in Fig. 3. The effective skin thickness and diffusion coefficient, with a 68% confidence interval, were estimated with the mathematical model and are listed in Table III. Using these parameter

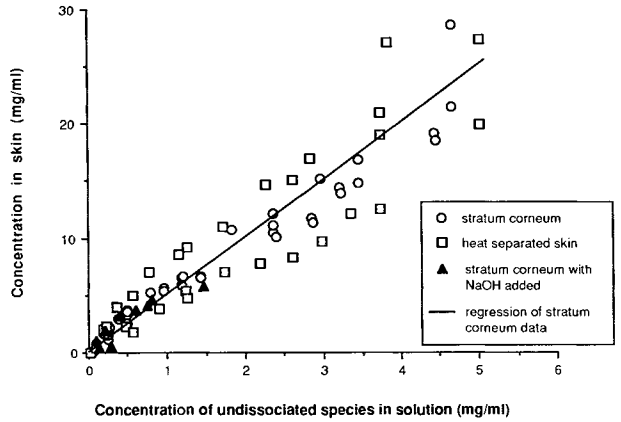

Fig. 2. Partitioning of benzoic acid between distilled water and separated skin

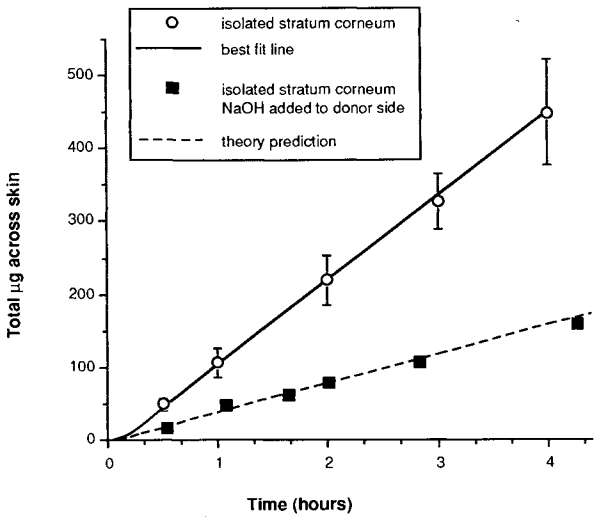

Fig. 3. Experimental data and mathematical calculations of accumulation in receiving chamber as a function of time for isolated stratum corneum.

estimates, the mathematical model generated a permeation profile represented by the solid curve in Fig. 3. From the calculated partition coefficient and estimated values of effective diffusivity and skin thickness, a permeability of 0.031 ± 0.060 cm/hr for stratum corneum was calculated using Eq. (2) with a propagation-of-errors analysis. The effective skin thickness and diffusion coefficient are strongly influenced by the lag time, which for the case of benzoic acid, is close to zero and susceptible to large deviations with small data variation. However, the permeability calculation is not as susceptible to data variations, as seen in Table III.

The estimated value of 32 ± 41 µm for stratum corneum thickness was not statistically different from the actual measured value of 40 ± 10 µm. The similarity of actual and effective permeation thickness suggests that the permeating benzoic acid follows a nontortuous pathway through the skin.

Values for lag time and permeability of isolated stratum corneum were also calculated from a linear fit to the apparent steady-state accumulation of benzoic acid in the receiving chamber. These lag times and permeabilities as well as calculated values for effective diffusivity and skin thickness are listed in Table III. The values for skin thickness and permeability were nearly equal to those estimated using the nonlinear model because in these experiments the donor chamber depletion and receiving chamber accumulation were small and did not influence the permeation profile.

The influence of epidermis and dermis on the permeation of benzoic acid was investigated using heat-separated (n = 6; three skin sources) and split-thickness skin (n = 6; three skin sources). The effective diffusivity, skin thickness, lag time, and permeability for each skin type are listed in Table III. Although the effective diffusivity, skin thickness, and lag time varied with skin type, the differences were statistically insignificant. Similar permeability values for stratum corneum, heat-separated, and split-thickness skin signify that the epidermis and dermis contributed no additional resistance and that the stratum corneum was the rate-limiting barrier to the permeation of benzoic acid through

Table III. Analysis of Benzoic Acid Permeation Through Stratum Corneum

	D_m (cm²/hr) $\times 10^5$	h (μm)	P (cm/hr)	t_{lag} (min)
Linear regression of stratum corneum permeation data[a]	10 ± 26	26 ± 40	0.030 ± 0.003	4.1 ± 6.3
Maximum-likelihood parameter estimation[b]				
Stratum corneum	2.1 ± 2.9	32 ± 41	0.031 ± 0.060	4.8 ± 11
Heat-separated skin	5 ± 10	70 ± 140	0.031 ± 0.089	11.2 ± 39
Split-thickness skin	8.0 ± 7.8	110 ± 100	0.034 ± 0.044	15.7 ± 24.7

[a] The linear regression has 68% confidence intervals for lag time and permeability; the other parameters have deviations calculated from propagation of errors.

[b] The nonlinear model has 68% confidence intervals for skin thickness and diffusion coefficient; the other parameters have deviations calculated from propagation of errors.

human skin. These permeation results also provide good evidence that the skin separation techniques did not alter the barrier function.

The stratum corneum was analyzed for benzoic acid after completing the permeation experiments as a confirmation of the partition coefficient value. At steady-state permeation, the content of benzoic acid in the stratum corneum should be one half the value of fully loaded stratum corneum. For a donor concentration of 4.8 mg/ml undissociated benzoic acid in water and a partition coefficient of 4.8, the fully loaded concentration should be 23.0 ± 0.5 mg/ml. At the end of the permeation experiments, the benzoic acid concentration in the stratum corneum was measured to be 12.2 ± 1.5 mg/ml (n = 6), compared with 11.5 mg/ml predicted from one-half of the predicted fully loaded value.

The assumption that the ionic species does not significantly permeate across skin was tested by adding sodium hydroxide to solutions of benzoic acid to significantly increase the percentage of dissociated species. Partition coefficients were measured between stratum corneum and pH adjusted solutions of benzoic acid (about 70% dissociation). Figure 2 includes these data designated as pH adjusted. When plotted as the concentration of benzoic acid in skin versus the concentration of undissociated benzoic acid in solution, the results from the pH adjusted experiments follow the results from experiments with no added base, supporting the hypothesis that only the undissociated species partitions into the skin.

Benzoic acid permeation was measured with sodium hydroxide added to the benzoic acid so that at 35°C, 65% of the benzoic acid would be dissociated (0.267 M NaOH and 5.0 mg/ml benzoic acid). Assuming that only the undissociated species partitions into the skin, the mathematical model generated the permeation profile represented by the dashed curve in Fig. 3. The experimental data are also shown in Fig. 3, and it is evident that the theory predicted the experimental permeation, supporting the hypothesis that only the undissociated species of benzoic acid partitions into and permeates across the skin.

In other experiments, we studied the effect that storing separated skin in distilled water at 4°C has on permeability. Skin from a single source (n = 3 for each case) was separated and used in permeation experiments after 1 day, 1 week, and 2 weeks of storage. Figure 4 shows that after 1 week of storage, the permeability of heat-separated skin increased 36%, whereas the permeability of stratum corneum remained essentially constant. After 2 weeks of storage, the permeabilities of stratum corneum and heat-separated skin were comparable and increased about 50% over their permeability after one day of separation. These results suggest some skin degradation under these storage conditions and that permeation experiments should be conducted soon after skin separation.

CONCLUSIONS

The objective of this study was to obtain physicochemical parameters from *in vitro* experiments that describe the controlling percutaneous absorption of benzoic acid across human skin. Skin–vehicle partition coefficients were measured and reported on a volume-to-volume basis. Permeation experiments were mathematically modeled including depletion in the donor chamber and accumulation in the receiving chamber. The apparent diffusion coefficient and skin thickness were obtained by fitting averaged permeation data to the non-steady-state mathematical model. Permeabilities were calculated from the measured partition coefficient and regressed values for the diffusion coefficient and thickness.

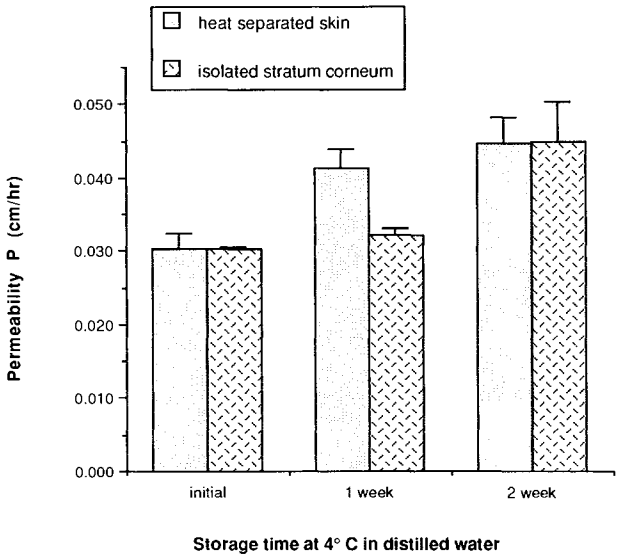

Fig. 4. Effect of storage time on the permeability of benzoic acid across isolated stratum corneum and heat-separated skin.

For benzoic acid, the stratum corneum–water partition coefficient was measured to be 4.8 ± 0.1, and the stratum corneum and epidermis had a nearly equal affinity for benzoic acid. For hydrated stratum corneum, the theoretical model calculated an effective thickness of 31 ± 41 μm, in comparison to a measured thickness of 40 ± 10 μm, and an apparent diffusion coefficient of 2.1 ± 2.9 × 10^{-5} cm^2/hr. The effective permeability was computed to be 0.031 ± 0.060 cm/hr. Differences between the permeabilities measured across isolated stratum corneum, heat-separated, and split-thickness skin were insignificant, indicating that the stratum corneum was the rate-limiting barrier for the permeation of benzoic acid across human skin. In addition, the skin separation techniques did not alter the barrier function of the stratum corneum.

Additional experiments measured the effect of storage time and benzoic acid dissociation. Skin permeability to benzoic acid increased when separated skin was stored at 4°C in distilled water for 1 to 2 weeks. Partition and permeation experiments with human skin indicated that only the undissociated species of benzoic acid is transported through the skin.

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NOMENCLATURE

A	Area of permeation (cm^2)
C	Concentration of permeating solute (μg/cm^3)
D	Effective diffusion coefficient (cm^2/hr)
h	Effective skin thickness (cm)
ΔH	Heat of dissociation (gcal/gmole)
K	Partition coefficient
M	Cumulative amount of solute passing through the membrane (μg)
P	Permeability coefficient (cm/hr)
t	Time (hr)
V	Volume of chamber (cm^3)
X	Distance through skin (cm)

Greek

ρ	Density (g/cm^3)

Subscripts

D	Donor chamber or vehicle
lag	Lag time for permeation
m	Skin
R	Receiving chamber
sc	Stratum corneum

Superscript

0	At time zero

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