Proc. Natl. Acad. Sci. USA
Vol. 94, pp. 1562–1567, February 1997
Pharmacology

Characterization of the permeability barrier of human skin *in vivo*

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Communicated by Robert Langer, Massachusetts Institute of Technology, Cambridge, MA, November 7, 1996 (received for review August 9, 1996)

ABSTRACT **Attenuated-total-reflectance Fourier-transform-infrared spectroscopy has been used to rapidly and noninvasively quantify *in vivo* the uptake of a chemical into the outermost, and least permeable, layer of human skin (the stratum corneum). The objective of the experiment was to develop a general model to predict the rate and extent of chemical absorption for diverse exposure scenarios from simple, and safe, short-duration studies. Measurement of the concentration profile of the chemical in the stratum corneum, and analysis of the data using the unsteady-state diffusion equation, enabled estimation of the permeability coefficient and calculation of the time required to achieve maximal transdermal flux. Validation of the spectroscopic technique employed was established, and quantitation of chemical uptake into the stratum corneum was confirmed independently using trace amounts of radiolabeled chemical in conjunction with liquid scintillation counting and accelerator mass spectrometry. The results presented have pharmacological and toxicological implications, as the technology lends itself both to the prediction of transdermal drug delivery, and the feasibility of this route of administration, and to the assessment of risk after dermal contact with toxic chemicals.**

The principal function of the skin is to act as a barrier to the insensible loss of tissue water. This objective is achieved by the stratum corneum (SC), the outermost layer of the epidermis, a lipid–protein biphasic structure, having a thickness of only 10–20 μm on most surfaces of the body. The excellent diffusional resistance of the SC makes the transdermal delivery of drugs at best difficult and frequently impossible. On the other hand, the SC protects against the dermal exposure of the organism to toxic chemicals. Nevertheless, there remains an important pharmaceutical need to reliably predict the topical and/or transdermal bioavailability of cutaneously applied drugs, and there is a crucial regulatory requirement for the accurate estimation of risk when inadvertent contact occurs between a potentially harmful substance and the skin.

Because of the transport rate-limiting ability of the SC, it is reasonable to suppose that, from a chemical's concentration profile across the membrane, even after a relatively short exposure, all necessary information relating to permeability can be deduced. In this regard, previous research has involved application of radiolabeled chemicals followed by SC removal using repeated adhesive tape-stripping, and analysis of the tape-strips by liquid scintillation counting (1). If the dose is sufficiently large, on the other hand, and a suitable extraction procedure is available for the chemical, more conventional analytical tools (e.g., HPLC) can be used. While these approaches have been used in humans, neither is particularly satisfying because of the need to expose the subjects to a significant amount of radioactivity or to a possibly excessive quantity of the chemical itself.

Recently, attenuated-total-reflectance Fourier-transform-infrared (ATR-FTIR) spectroscopy has been exploited as a fast and versatile tool to study SC barrier function *in vivo*, in humans (2). It has been used, for example, to measure the water content of the SC (3, 4), to study the action of permeation enhancers, and to track the diffusion of a model compound [4-cyanophenol, CP; molecular weight = 119, log ($K_{\text{octanol/water}}$) = 1.6] as a function of the vehicle used (5, 6). In short, it provides a noninvasive means to track diverse, nonvolatile molecules possessing characteristic vibrational modes and suitably distinct IR absorbances. In the research described here, the permeation of CP was monitored after a brief exposure (15 min) to an aqueous solution of the chemical. From the concentration profile obtained, and the theoretical analysis of the data, transport after longer exposure times was predicted and confirmed. The experiments yielded the characteristic transport parameter (ratio of the diffusivity to the diffusion pathlength squared) for CP diffusion across the SC, and the partition coefficient of the chemical between SC and water.

The robustness of the ATR-FTIR approach was checked against standard liquid scintillation counting of radiolabeled CP. The results also have been compared with those obtained by using accelerator mass spectrometry (AMS) for the analysis of CP (7, 8). Although AMS also requires the use of ^{14}C-labeled CP, the "dose" of radioactivity is extremely small (and close to "risk-less") due to the exquisite sensitivity of the method. It appears, therefore, that ATR-FTIR and AMS can provide complementary techniques capable of monitoring the skin permeability of multiple and diverse classes of chemicals.

THEORY

Transport through the SC generally is considered to follow Fickian diffusion through a simple, homogeneous membrane (9). Under the boundary conditions of the experiments performed, i.e.,

(*i*) at $x = 0$, $C = C_{x=0} = K{\cdot}C_{\text{veh}}$, $t \geq 0$,
(*ii*) at $x = L$, $C = 0$, $t \geq 0$, and
(*iii*) at $0 < x < L$, $C = 0$, $t = 0$,

the concentration profile of the chemical [i.e., $C(x)$ as a function of position x and time t] is given by the well known solution to Fick's Second Law (10):

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Abbreviations: SC, stratum corneum; ATR-FTIR, attenuated-total-reflectance Fourier-transform infrared; CP, 4-cyanophenol; AMS, accelerator mass spectrometry.

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$$C(x) = KC_{\text{veh}}\left\{1 - \frac{x}{L}\right\} - \sum_{n=1}^{\infty} \frac{2}{n\pi} KC_{\text{veh}} \sin\left(\frac{n\pi x}{L}\right) \exp\left(\frac{-Dn^2\pi^2 t}{L^2}\right), \quad [1]$$

where $C_{x=0}$ is the permeant's concentration at the skin surface (i.e., $x = 0$), L is the diffusion pathlengh of the chemical across the SC, and D is its diffusivity. It is assumed that the chemical equilibrates rapidly between the vehicle and the SC at the skin surface, so that $C_{x=0} = K{\cdot}C_{\text{veh}}$, where K is the SC/vehicle partition coefficient of the chemical, the concentration of which in the vehicle is C_{veh}. The validity of Eq. **1** also assumes that (*i*) all transport of chemical across the SC is by passive diffusion, (*ii*) the vehicle in which the chemical is presented to the SC does not modify the membrane or act as a carrier for the compound, and (*iii*) that no other skin layers contribute to the total barrier. The rate per unit area at which chemical emerges from the dermal face of the SC (i.e., at $x = L$) is given by $-D(\partial C/\partial x)_{x=L}$ and can be expressed by differentiation of Eq. **1** (10). Equally, integration of this rate over time yields the cumulative amount (Q_t) of chemical that has diffused through the SC as a function of time.

After exposure of human skin *in vivo* to an aqueous solution of CP (of known C_{veh}) for 15 min, ATR-FTIR analysis of the sequentially tape-stripped SC yielded values of $C(x)$ as a function of position. The data were fit to Eq. **1** to yield values of D/L^2 [obtained from the decay of $C(x)$ as a function of x] and K (from the intercept at $x = 0$). The D/L^2 parameter has dimensions of $(\text{time})^{-1}$ and can be thought of as a first-order rate constant for diffusion across the membrane, the reciprocal of which corresponds to a characteristic transport time. These parameters then were used to predict the concentration profile after a 1-h exposure, and the experimental data were compared with this estimate. It was also possible then, by choosing reasonable values for L, to calculate the flux across the SC, and the cumulative amount permeated, as a function of time, and the CP permeability coefficient ($K_p = KD/L$) across the SC.

MATERIALS AND METHODS

Materials. CP (4-hydroxybenzonitrile) and [^{14}C]CP were purchased from Aldrich and Moravek Biochemicals (Brea, CA), respectively. Aqueous solutions of CP were prepared with deionized water. The system used for the cutaneous application of CP solutions comprised a Soft-wick-IV-sponge (8 × 2 cm, Johnson and Johnson Medical, Arlington, TX), a polyester film (9 × 3 cm, Scotchpak, 3M, St. Paul, MN), and a nonocclusive transparent dressing (10 × 12 cm, Tegaderm 1626, 3M). 3M Book Tape 845 was used for sequential SC removal by stripping.

Instrumentation. ATR-FTIR spectra were recorded using a Nicolet 520 FT-IR spectrometer equipped with a Balston BFS-400 FT-IR air purifier (Balston, Lexington, MA) and a liquid nitrogen-cooled mercury–cadmium–telluride detector. The sampling compartment (Contact Sampler, Spectra Tech, Stamford, CT) was a horizontal internal reflection accessory with an IR transmitting crystal of high refractive index. An evanescent wave penetrated into the sample in contact with the crystal, producing a spectrum of the sample. In this study, a zinc selenide crystal (7 × 1 cm, 45°) with a refractive index of 2.4 at 1000 cm^{-1}, a density of 5.27 $\text{g}{\cdot}\text{cm}^{-3}$, and a transmission range of 20,000–650 cm^{-1}, was used. All spectra obtained represented an average of 100 scans, over the wavelength range of 4000–600 cm^{-1}, and required 3–4 min for acquisition.

Method Validation. Quantification of CP on the adhesive tape to be used for progressive removal of the SC involved integration of the C≡N stretching vibration absorbance in the spectral region between 2250 and 2200 cm^{-1}; importantly, this wavelength range contained little interference from the adhesive matrix (6). The accuracy of the detection method was tested by spiking the adhesive matrix with low, medium, and high concentrations (3.2, 12.7, and 123.8 nmol/cm^2, respectively) of CP. The CP on the adhesive matrix then was analyzed directly by ATR-FTIR. The adhesive tape was placed (adhesive side down) onto the zinc selenide crystal, and a spectrum was recorded. Repeatability and reproducibility measurements were performed for each concentration (11).

A calibration curve was obtained over the CP concentration range identified. The limit of detection and the limit of quantification were assessed as the ratios $3SD_b/S$ and $9SD_b/S$, respectively, where SD_b is the standard deviation of the absorbance from a blank tape, and S is the slope of the absorbance versus CP concentration calibration curve (11).

Because of the potential attenuation of the IR signal on the tape-strips by the removed SC, the effect of the presence of tissue on the ATR-FTIR measurements was assessed by analyzing tapes contaminated both with various CP concentrations (8–248 nmol/cm^2) and with typical amounts of stripped SC (6–84 $\mu\text{g/cm}^2$).

***In Vivo* Percutaneous Absorption.** *In vivo* studies were approved by the Committee on Human Research at the University of California, San Francisco, and were conducted in two groups of healthy volunteers ($n_1 = 3$ and $n_2 = 4$, aged 28 ± 2 years) with no history of dermatologic disease. The ventral forearm surface was used for all experiments.

In the first part of the study, 500 μl of a saturated aqueous solution of CP (196 mM, 6.125 $\mu\text{mol/cm}^2$) was applied to the skin for either 15 min or 1 h. At the end of the treatment period, the delivery system was removed, and the skin surface was gently dried. The SC at the treated site then was progressively removed by repeated adhesive tape-stripping (20 tape strips were typically removed). The concentration profile of CP across the SC and the total concentration taken up into the membrane were determined as follows. First, an ATR-FTIR spectrum of each tape-strip was immediately recorded (to ensure that CP in the SC had no opportunity to diffuse into the adhesive matrix of the tape) as described above for the validation procedure, and the mass of SC on each tape-strip was determined by weighing on a high-precision (sensitive to 10 μg) balance (Sartorius BP 210D, Sartorius AG, Goettingen, Germany). The amount of CP on each tape-strip then could be evaluated from the calibration curve. Assuming a SC density of 1 $\text{g}{\cdot}\text{cm}^{-3}$ (12), the mass removed was converted to a volume, and in turn, as the area exposed was fixed, to an effective thickness of SC per tape-strip. Thus, this enabled CP concentrations in nmol/cm^2 on each tape-strip to be reexpressed in units of molarity.

In the second set of experiments, the 15-min exposure procedure was repeated in different volunteers using the same CP solution but this time "spiked" with 10,000 dpm (630 dpm/cm^2) of ^{14}C-radiolabeled chemical (specific activity = 45 μCi/mol; 1 Ci = 37 GBq). At the end of the treatment period, the system was removed, the skin surface was cleaned, and the tape-stripping procedure was repeated as described above (including ATR-FTIR analysis and determination of SC mass removed). Subsequently, each tape-strip was placed in a scintillation vial containing 15 ml of methanol for 48 h, at which point the radioactivity was determined by liquid scintillation counting. Control experiments showed that the extraction procedure efficiency was 92% ± 8%. Furthermore, 1 ml of each methanolic solution was reserved for analysis by AMS (7, 8), which is an isotope-ratio mass spectrometry method of exquisite sensitivity (1 amol/mg of carbon for ^{14}C) (7, 8). AMS measurements were performed using standard sample preparation techniques at The Center for Accelerator Mass Spectrometry (Lawrence Livermore National Laboratory, Livermore, CA).

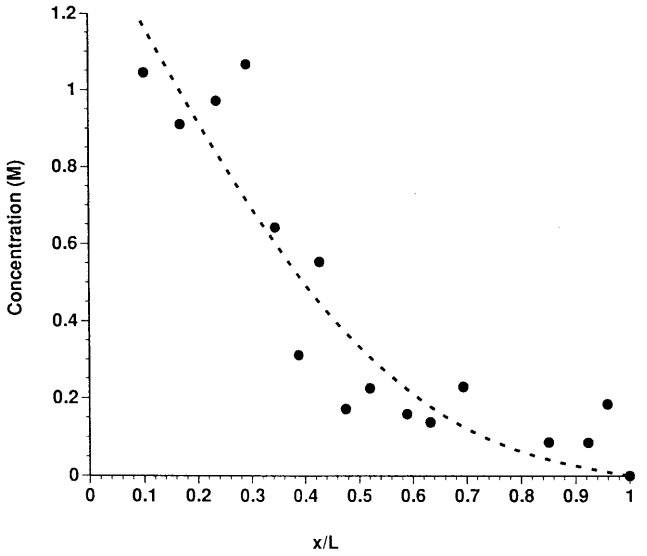

FIG. 1. The concentration profile of CP transport across human SC (subject A) *in vivo* after exposure of the skin to an aqueous solution of the chemical for 15 min. The values on the abscissa (x/L) are calculated from the ratio $(\Sigma_{i=1}^{i} M_i)/(\Sigma_{i=1}^{n} M_i)$, where M_i is the SC mass removed by the ith tape-strip and $\Sigma_{i=1}^{n} M_i = M_T$, the total SC mass removed by all n tape-strips combined. Nonlinear regression was used to obtain the best fit of Eq. **1** (dashed line; $r^2 = 0.84$) to the data. From this analysis, it can be deduced that $D/L^2 = 9.90 \times 10^{-5}$ s^{-1} and $K = 5.5$.

RESULTS

Method Validation. In previous ATR-FTIR experiments, the relative concentration of CP in the SC was determined by recording spectra directly from each newly exposed surface of the membrane during the tape-stripping procedure (6). Here, on the other hand, the absolute quantity of CP as a function of SC depth was determined by measuring an ATR-FTIR spectrum of each of the sequential tape-strips. To calibrate this approach, a number of experiments were performed. First, repeatability and reproducibility were examined to ensure that the contact between the tape-strips and the ATR-FTIR crystal was constant (11). High, medium, and low concentrations of CP were applied to different tape-strips, and the C≡N absorbances were determined by successively analyzing each tape-strip 10 times. At the low and medium concentrations, the relative standard deviation values of the absorbances were ≤8%; at high concentration, they were ≤1%. The inter-experimental relative standard deviation was within the range of 7–13%.

The relationship between CP absorbance and the CP concentration (in nmol/cm^2) on the tape-strips was linear, but was modified by the presence of SC, according to the relationship

$$\Lambda = 0.004[\mathrm{CP}]\cdot\exp(-0.014M), \qquad [2]$$

where Λ is the C≡N area under the absorbance from CP on the tape-strip and M is the mass (μg/cm^2) of SC on the tape-strip.

The limit of detection and the limit of quantification of CP were 0.8 and 2.54 nmol/cm^2, respectively.

***In Vivo* Percutaneous Absorption Data.** Fig. 1 shows the ATR-FTIR assessed concentration profiles of CP across human SC *in vivo* (for subject A) after a 15-min exposure of the skin to an aqueous solution of the chemical. The dashed curve represents the best fit of Eq. **1** to the data obtained. Similar results from three subjects allowed values of D/L^2 and K to be obtained (see Table 1).

From the 15-min exposure results, the mean value of D/L^2 predicts that the classic diffusional lag time for CP transport across the SC ($L^2/6D$) (10) is about 0.5 h. It may be predicted, therefore, that a linear concentration profile (i.e., steady-state diffusion) of CP should be established by prolonging the exposure to 1.0–1.5 h (10). Exposures of 1-h duration therefore were performed to test this deduction, and Fig. 2 illustrates the agreement between experiment and prediction for one subject. Comparable findings were achieved in the other subjects (data not shown).

The major intended application of the experimental protocol and data analysis is to allow prediction of the flux (J_t) and cumulative amount (Q_t) of chemical diffusing through the SC *in vivo* as a function of time. Such information, of course, would be invaluable for both evaluating the feasibility of topical or transdermal drug delivery and assessing the risk associated with dermal exposure to a toxic chemical. As mentioned above, expressions for J_t and Q_t are easily derived from Eq. **1** (10). However, the experimental data obtained and the analysis employed require that the SC thickness (more precisely, the diffusion pathlength of the chemical across the SC) be known so that J_t, Q_t, and, ultimately, K_p and the steady-state flux (J_{ss}) can be unequivocally determined. The most straightforward approach to obtaining SC thickness is to measure transepidermal water loss as a function of tape-stripping and to use a simple data analytical procedure that has

Table 1. Measured, fitted, and predicted parameters characterizing CP transport across human SC *in vivo*, after application of an aqueous CP solution for 15 and 60 min

		15-min fitted parameters					60-min fitted parameters				60-min predicted parameters	
Subject	[CP] in SC at 15 min,* M	K†	D/L^2,† 10^5 s^{-1}	$C_{x=0}$,‡ M	t_{lag},§ min	[CP] in SC at 60 min,* M	K¶	$C_{x=0}$,‡ M	J_{ss},‖ nmol·cm^{-2}·s^{-1}	10^7 K_p,** cm·s^{-1}	J_{ss},†† nmol·cm^{-2}·s^{-1}	$10^7 K_p$,‡‡ cm·s^{-1}
A	0.40	7.3	9.9	1.43	28.1	0.40	4.9	0.95	0.14	7.1	0.21	10.7
B	0.33	5.5	9.3	1.07	29.9	0.61	7.1	1.40	0.20	10.2	0.15	7.7
C	0.61	12.4	7.0	2.42	39.7	0.87	10.1	1.98	0.21	10.7	0.25	12.8
Mean	0.45	8.4	8.4	1.64	32.5	0.63	7.4	1.44	0.18	9.4	0.20	10.4
SD	0.15	3.6	1.5	0.70	6.2	0.24	2.6	0.52	0.04	1.9	0.05	2.6

*Measured experimentally.
†From the fit of Eq. **1** to the 15-min *in vivo* data.
‡$C_{x=0} = K\cdot C_{veh}$.
§$t_{lag} = L^2/6D$.
¶From the fit of the steady-state form of Eq. **1** to the 60-min *in vivo* data.
‖Calculated from the gradient of the fitted *in vivo* data to the steady-state form of Eq. **1**, assuming $L = 15$ μm.
**$K_p = J_{ss}/C_{veh}$.
††$J_{ss} = (K\cdot D/L)\cdot C_{veh}$ using K and D/L^2 values from the 15-min exposure experiments, and assuming $L = 15$ μm.
‡‡$K_p = K\cdot D/L$ using K and D/L^2 values from the 15-min exposure experiments, and assuming $L = 15$ μm.

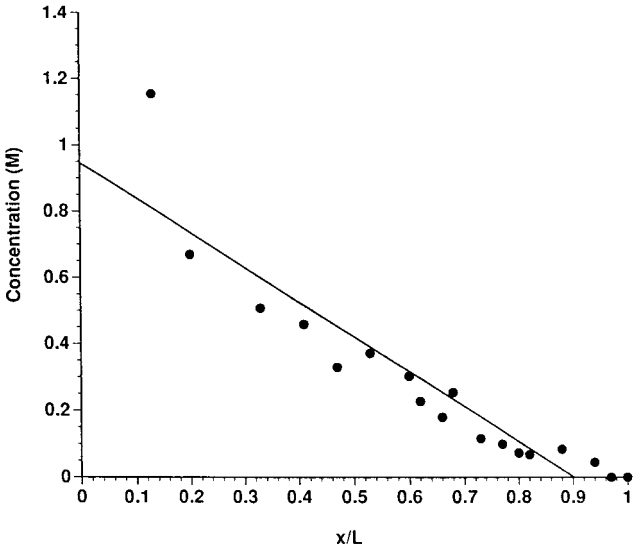

FIG. 2. The concentration profile of CP across human SC (subject A) after exposure of the skin to an aqueous solution of the chemical for 1 h. The values on the abscissa (x/L) are calculated as described for Fig. 1. The slope and intercept of the line of linear regression through the data are −1.05 M and 0.95 M, respectively. The values predicted from the 15-min exposure data (Fig. 1) are −1.43 M and 1.43 M, respectively.

recently been developed in our laboratory (13). It should be noted, though, that this method does not account for any tortuosity associated with (for example) an intercellular pathway of permeation across the membrane (14). Typically, we have found L to be on the order of 10–20 μm, values in complete agreement with previous work (15), and we have chosen, somewhat arbitrarily, therefore, to use $L = 15$ μm to calculate apparent *in vivo* values of J_{ss} and K_p for CP (Table 1). To illustrate the evolution of J_t and Q_t, as a function of time, Figs. 3 and 4 plot these respective functions based upon the average D/L^2 value observed experimentally (8.7×10^{-5} s^{-1}), and three different combinations of D and L: (*i*) $D = 8.7 \times 10^{-11}$ cm^2·s^{-1}, $L = 10$ μm; (*ii*) $D = 1.9 \times 10^{-10}$ cm^2·s^{-1}, $L = 15$ μm; and (*iii*) $D = 3.5 \times 10^{-10}$ cm^2·s^{-1}, $L = 20$ μm.

Last, in a different group of subjects, the total uptake of CP into human SC *in vivo* was determined not only by ATR-FTIR but also, using small amounts of ^{14}C-labeled CP, by liquid scintillation counting, and AMS (7, 8). The agreement between the results obtained is shown in Table 2 and Fig. 5.

DISCUSSION

The utility of the approach described rests on the fact that the SC is the principal barrier to molecular transport into the body via the skin (16). Therefore, knowledge of the flux through the SC, and/or the cumulative amount permeating, as a function of time, provides information essential for the optimization of topical and transdermal drug therapy and for the evaluation of potential toxicity resulting from skin exposure to dangerous chemicals.

The fact that the procedures used are very amenable to human, *in vivo*, experimentation gives the data obtained considerable additional weight over those from either animal or *in vitro* studies. In the latter cases, there always remain questions relating to relevance and the extent to which correlation with results that would be obtained in humans may be expected. Although ATR-FTIR will not be useful for all chemicals (due, e.g., to their volatility, or to the lack of suitably distinct or significant IR absorbance), the excellent correlation observed here between IR and AMS results suggests that the two procedures may be nicely complementary and permit

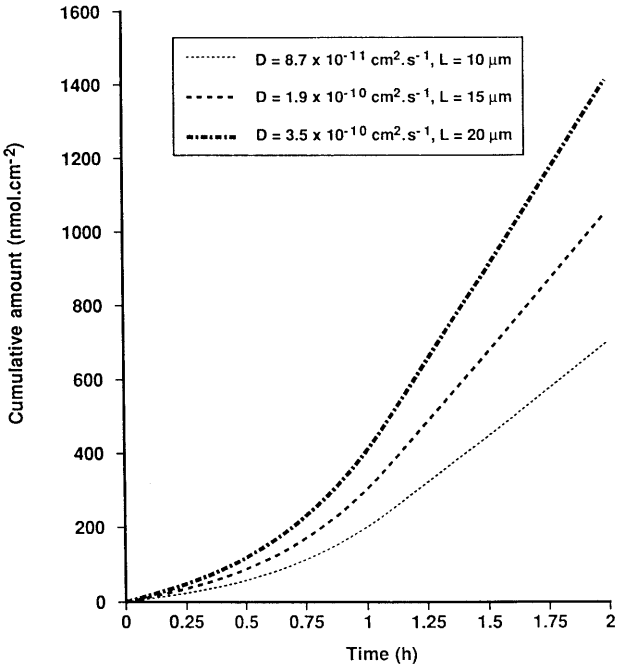

FIG. 3. Prediction of the cumulative amount (Q_t) of CP permeating the skin as a function of time after exposure to an aqueous solution of the chemical. The calculated curves use the mean values of K and D/L^2 derived from the 15-min exposure experiments and the three different combinations of D and L as indicated.

chemicals of very diverse physical and pharmacological properties to be examined. The exquisite sensitivity of AMS means that the level of ^{14}C radioactivity to which the human subjects are exposed is vanishingly small, to the extent that the risk associated with the experiment is well below that which causes even the mildest concern (17). While AMS at present is a

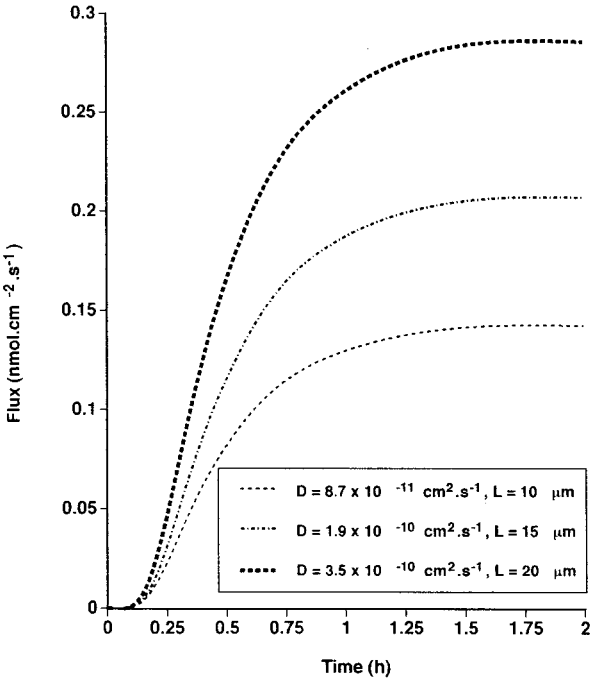

FIG. 4. Prediction of the flux (J_t) of CP permeating the skin as a function of time after exposure to an aqueous solution of the chemical. The calculated curves use the mean values of K and D/L^2 derived from the 15-min exposure experiments, and the three different combinations of D and L as indicated.

Table 2. CP concentrations in 20 tape-strips of SC measured by ATR-FTIR spectroscopy, liquid scintillation counting (LSC) and AMS after a 15-min exposure to an aqueous solution of the chemical

	Applied concentration		Total amount in SC,* nmol·cm^{-2}·mg^{-1}		
Subject	mM	nmol·cm^{-2}	ATR-FTIR	LSC	AMS
D	196	4410	84	62	129
E	196	4410	40	32	37
F	196	4508	64	51	69
Mean			63	48	78
SD			22	15	47

The average total amounts of CP in the stratum corneum as determined by the three methods were statistically indistinguishable ($P > 0.05$, Kruskal–Wallis test).

*In a fourth subject, for whom AMS data were not available, the ATR-FTIR and LSC measurements were 66 and 40 nmol·cm^{-2}·mg^{-1}, respectively. For a fifth subject, to whom a lower CP concentration (149 mM) was applied, ATR-FTIR, LSC, and AMS values were 13, 15 and, 26 nmol·cm^{-2}·mg^{-1}, respectively.

technique of limited, general accessibility, "bench-top" spectrometers for routine biomedical research are clearly "in the pipeline" and are expected to be routinely available soon (7, 8). A further important advantage is that the tape-stripping procedure can be performed in a time period much shorter than that for any significant diffusion of the chemical in the SC to occur.

It is appropriate to comment upon the quality of the results produced in this study, relative to earlier work in the percutaneous field, and the agreement between the observations made here with those from other investigations. The variability in the amount of CP taken up into the SC after 15- and 60-min exposures reflects a range that is certainly within that observed in other *in vivo* experiments. For example, when CP permeation in man was measured by the "classic" technique of solvent deposition of a (relatively) large dose of radiolabeled chemical, followed by monitoring of ^{14}C urinary excretion (18), the coefficient of variation associated with the cumulative amount absorbed was 13% to 50% depending upon the exposure conditions (19). However, these earlier studies yielded almost no information about the kinetics of the percutaneous absorption penetration *per se* due to the fact that quantification of the material absorbed is made so "distal" to the site of uptake. Measurements in the blood are better, of course, but such procedures are invasive and expensive and demand (usually) high chemical doses and sophisticated (and specific) analytical tools (18).

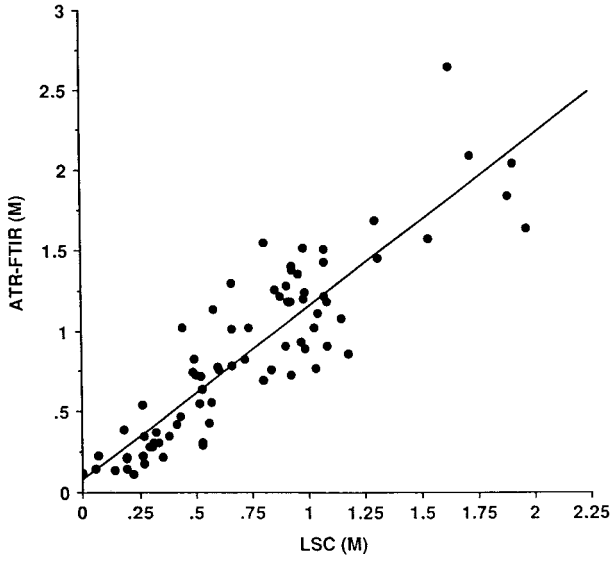

FIG. 5. Correlation between the concentrations of CP in the SC, after a 15-minute exposure to an aqueous solution of the chemical, as measured by ATR-FTIR spectroscopy and by liquid scintillation counting (LSC). The accumulated data from four different subjects (78 measurements) are shown. The line of linear regression drawn through the data is $y = 1.07\,x + 0.08$ ($R^2 = 0.796$, $P < 0.0001$). The values of the slope and intercept are not significantly different from 1 and 0 ($P > 0.05$), respectively.

The derived physicochemical transport parameters (K and D/L^2) from this work are, of course, only as good as the model used to analyze the results. If the model is poorly chosen, or unreasonable, the parameters with which it is characterized will be useless. An overview of the field, however, clearly reveals a consensus that the SC diffusion process is passive and that Fick's laws are generally adequate for an initial level of interpretation (9). Nevertheless, preliminary *in vitro* and/or animal experiments remain important for estimating diffusional parameters in more complex situations involving, for example, interaction of the permeant with structures along the transport route. These experiments can provide, therefore, valuable estimates of the time required to reach steady-state, and thereby serve usefully in the design of subsequent *in vivo* experiments. Debate continues about the pathway(s) of molecular permeation and contributes to the uncertainty, alluded to above, with respect to the diffusion pathlength across the SC (14). This means that essentially all reported diffusivities in the literature, including those that may be derived from the data presented here, must be prefaced by the word "apparent."

Although D/L^2 for CP in human skin has not been explicitly measured before, the average value for three subjects reported here [8.7 ($\pm$1.5) $\times$ 10^{-5} s^{-1}] can be compared favorably to values for methyl nicotinate ($\approx$20 $\times$ 10^{-5} s^{-1}), which were deduced from completely different *in vivo* experimental procedures (20, 21). Both CP and methyl nicotinate are reasonably small nonelectrolytes (molecular weight < 140) of somewhat lipophilic nature (octanol–water partition coefficients of between 10 and 100, melting points < 100°C). The SC–water partition coefficient ($K \approx 8$), which has been deduced, agrees very closely with that determined *in vitro* (range 6–11) by equilibrating isolated sheets of human SC with aqueous CP solutions of different concentration (22). Taken together, the overlap between the derived values of D/L^2 and K reported here with those found by other investigators elsewhere using quite distinct methodologies, lends considerable support to the reasonableness of the model employed for data analysis.

It also may be noted that the calculated CP permeability coefficient ($K_p \approx 10^{-6}$ cm·s^{-1}), based on an assumed value of $L = 15$ μm, is quite consistent with that predicted by a completely theoretical model (23), which estimates K_p based upon a solubility-diffusion algorithm using the octanol–water partition coefficient (P) and the molecular weight of the chemical [$\log K_p$ (cm·s^{-1}) $= -6.3 + 0.71 \cdot \log P - 0.0061 \cdot M_r$]. For CP, $\log P = 1.6$, and $M_r = 119$, yielding a predicted K_p of 1.3 $\times$ 10^{-6} cm·s^{-1}. From this agreement, we may further conclude that the extrapolation from the short-exposure results to the steady-state situation is reasonable.

In conclusion, therefore, facile experimental methodology, applicable *in vivo* in humans (with real levels of skin hydration) and straightforward data analytical procedures have been described and validated for their ability to provide (*i*) valuable measurements of skin permeation, and (*ii*) apparently predictive tools for the quantification of chemical transport across the SC barrier under diverse exposure scenarios. Applications of the approach in pharmacology and toxicology may be envisaged.

F.P. thanks Hoffmann–La Roche, the Philippe Foundation, Prof. P. Agache, and Prof. Ph. Humbert (Centre de Recherche sur le Tégument, France) for financial support. This research was supported by

the National Institutes of Health (HD 23010), the U.S. Air Force Office of Scientific Research, and the U.S. Environmental Protection Agency.

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