International Journal of Pharmaceutics, 39 (1987) 149–156
Elsevier

IJP 01320

Development of model membranes for percutaneous absorption measurements. I. Isopropyl myristate

Jonathan Hadgraft and Geoffrey Ridout *

Department of Pharmacy, University of Nottingham, Nottingham U.K.

(Received 31 March 1987)
(Modified version received 10 May 1987)
(Accepted 16 May 1987)

Key words: Percutaneous absorption; Rotating diffusion cell; Artificial lipid membrane; In vitro skin model; Isopropyl myristate; Structure–activity relationship

Summary

Studies have been conducted to test the validity of an in vitro model for percutaneous absorption in humans. The system consists of an artificial lipid membrane, which mimics the epidermal barrier, supported in a rotating diffusion cell. The artificial membrane was formed with isopropyl myristate (IPM), a lipid chosen to be representative of those in the stratum corneum. The membrane resistance to penetration has been studied for a range of compounds with diverse physicochemical properties. A positive correlation between transport resistance and the IPM-aqueous partition coefficient is demonstrated. The penetration data obtained from the artificial system are compared with those measured using excised human cadaver skin as the membrane in glass diffusion cells. A reasonable correlation was found between the resistances to diffusion provided by the IPM membrane and by excised skin, although the magnitude of the former was 1000-fold lower than of the latter. However, the IPM membrane did not predict the relatively low resistance of excised human skin to isoquinoline and nicotine. It is suggested that although the artificial membrane described provides a reasonable model for percutaneous absorption, modifications should be made to improve the predictability of the system.

Introduction

The physicochemical factors that control percutaneous absorption in man have recently received much interest (Barry, 1983; Bronaugh and Maibach, 1985). A knowledge of these factors would enable prediction of the absorption characteristics of (a) drugs formulated as topical preparations, (b) candidates for transdermal delivery and (c) environmental and industrial toxinogens that contact the skin.

Many in vivo and in vitro experimental techniques have been employed in an attempt to elucidate the underlying mechanisms of percutaneous absorption (Maibach and Lowe, 1985). To gain a full knowledge of how a compound will interact with and permeate the skin, the most relevant data are obtained in vivo in man (Feldmann and Maibach, 1967). However, there are several reservations to this; there is a wide biological variability, the procedures are expensive and time-consuming, and must meet with ethical approval. Compounds with known toxicity cannot be tested in this way.

Present address: Departments of Pharmacy and Pharmaceutical Chemistry, University of California at San Francisco, San Francisco, CA 94143, U.S.A.
Correspondence: J. Hadgraft, The Welsh School of Pharmacy, UWIST, Cardiff CF1 3XF, U.K.

Because of the problems encountered with in vivo procedures, reliable to in vitro methodology is required. The in vitro techniques currently employed involve determination of the transport of chemicals across excised skin of either animal or human origin, or across artificial model membranes (Bronaugh and Maibach, 1985). The use of excised skin is subject to species and interindividual variation. To date, the skin of no one animal species has been shown to be a valid model of human skin. Thus, human skin, which has exhibited a correlation with in vivo data to a wide range of substances, is preferred. However, sources of excised human skin are limited. Artificial, or model, membrane systems offer an alternative to excised skin. These models have several advantages over biological systems; they are much more reproducible, easily manufactured and the membrane composition is easily manipulated.

Diffusion through the outermost skin layer, the stratum corneum, is accepted as the rate-limiting step in the percutaneous transport of most substances (Scheuplein, 1965). Therefore, to model skin successfully, the artificial membrane should mimic the barrier properties of human stratum corneum. The transport of many chemicals through a variety of model in vitro systems has been studied in an attempt to elucidate the underlying physical chemistry of percutaneous absorption. However, only a limited number of definitive structure–activity relationships have been produced (Flynn, 1985) and, to date, none of the in vitro systems used has been demonstrated to be optimal.

The objective of the work reported here was to test the validity of an in vitro model for percutaneous absorption in humans. The system consists of an artificial lipid membrane supported in a rotating diffusion cell (RDC). The compounds chosen as model skin penetrants (Table I) represented the diverse physicochemical properties that transdermally absorbed substances may possess. The compounds included an acid (salicylic acid (SA)), two bases (isoquinoline (IS) and nicotine (NI)), a lipophilic steroid (hydrocortisone (HY)), and an analogous series of barbiturates (amylobarbitone (AM), barbitone (BA), butobarbitone (BU) and phenobarbitone (PH)). The RDC was used to study the transport parameters of these compounds across a simulated epidermal barrier consisting of a membrane filter impregnated with isopropyl myristate (IPM), a liquid representative of skin lipids (Poulsen et al., 1968). The use of such a system, as a model for percutaneous absorption, has been extensively documented in the past. A positive correlation between RDC transport and in vivo human skin penetration data has been reported for a series of nicotinates (Albery and Hadgraft, 1979). The results have been analysed to determine whether any structure–activity relationship exists for permeation through this system. In order to determine the validity of the model, the resistance of the artificial membrane to each penetrant was compared with the corresponding values derived from a traditional in vitro diffusion method using excised human skin mounted in glass diffusion cells (Franz, 1975).

Materials and Methods

Materials

AM PH (both May and Baker); BA (Boots Company); BU (Sigma Chemicals Co.); HY (gift from Glaxo Group Research); IS (Koch-Light Laboratories); NI, SA, 1,2-dimethyldichlorosilane (2% in 1,1,1-trichloroethane) (all of AnalaR grade — BDH) and IPM (Croda Chemicals Ltd.) were used as received. Reagents used for HPLC analyses were glacial acetic acid (SLR grade, Fisons Reagents), acetonitrile and methanol (both of HPLC grade, Rathburn Chemicals Ltd.). Salts used for the preparation of the buffers were of GPR grade supplied by BDH. Cellulose nitrate membrane filters, 0.2 μm pore size, were obtained from Whatman. Distilled water from a Bibby Aquatron W4S all-glass still was used throughout.

Methods

Rotating diffusion cell studies

Transport across the lipid membrane and associated aqueous-organic interfaces was measured with a RDC, in which the thickness of stagnant diffusion layers on either side of the filter (Z_D) is

controlled by the cell rotation speed (Albery et al., 1976).

The membrane consisted of a 0.2 μm cellulose nitrate membrane filter that was first rendered hydrophobic by treatment with a solution of 1,2-dimethyldichlorosilane (2% in 1,1,1-trichloroethane) and was then saturated with IPM.

Ideally, the model should mimic the natural pH gradient that exists across human skin in vivo. A donor solution at pH 5.0 and a receptor at pH 7.4 would simulate the pH of the skin surface and the dermis respectively (Poulsen et al., 1968). However, to ensure that any effects due to ionisation were minimised, the pH of the donor and receptor phases used for IS, NI and SA were as given in Table 1. The donor solution for hydrocortisone contained 5% v/v ethanol as a cosolvent. The use of such concentrations of ethanol had previously been demonstrated not to influence permeation characteristics of IS and SA in a preliminary experiment. The initial donor phase concentrations of the penetrants studied are also given in Table 1. The volumes of the donor and receptor compartments were 30 and 300 ml, respectively. The concentration of diffusate in the receptor compartment was continuously monitored by using a flow-cell in conjunction with a Cecil 292 UV spectrophotometer set at the appropriate λ_{max} (Table 1) and a LKB Varioplex II peristaltic pump. All experiments were conducted at 32°C, a temperature representative of that of the skin surface. The apparent first-order rate constant for diffusate appearing in the receptor phase were determined as a function of cell rotation speed. Four rotation speeds were employed and the results reported for each compound were obtained using at least 5 different membranes.

Analysis of data

Providing sink conditions exist in the receptor phase, the flux (J) at which solute is transferred from the inner (donor) to the outer (receptor) compartment is given by Eq. 1:

$$J = (A \cdot C_i)\vec{R}_T \quad (1)$$

where A is the effective area of the membrane and C_i is the solute concentration in the donor compartment. $\vec{R}_T$ is the total resistance to solute permeation from the bulk of the donor to the bulk of the receptor, being the inverse of the overall permeability coefficient, K_p. $\vec{R}_T$ may be expressed by Eqn. 2.

$$\vec{R}_T = 2R_{SL} + 2R_I + R_M \quad (2)$$

(i) $2R_{SL}$ describes the resistance to transfer across the two stagnant diffusion layers and may be expressed as:

$$2R_{SL} = 1.286W^{-1/2} \times v^{1/6} \times D_{aq}^{-2/3} \quad (3)$$

where (in these experiments), W is the rotation speed (Hz), v is the kinematic viscosity and D_{aq} is the diffusion coefficient of the solute in the aqueous medium (Albery et al., 1976).

(ii) $2R_I$ describes the interfacial transfer resistance, the factor of 2 arising because there are two aqueous-organic interfaces.

TABLE 1

λ_{max} for the UV detection of the penetrants and the composition of the donor and receptor phases used in RDC experiments

Compound	λ_{max} (nm)	Donor phase	Receptor phase	Donor concentration
Amylobarbitone	240	pH 5.0 NaOH	pH 7.4 buffer	2.5 mM
Barbitone	240	pH 5.0 NaOH	pH 7.4 buffer	10.0 mM
Butobarbitone	240	pH 5.0 NaOH	pH 7.4 buffer	2.5 mM
Phenobarbitone	240	pH 5.0 NaOH	pH 7.4 buffer	2.5 mM
Hydrocortisone	248	Ethanol : water 5 : 95 ml	pH 7.4 buffer	1.0 mM
Isoquinoline	315	pH 7.4 NaOH	pH 7.4 NaOH	1.0 mM
Nicotine	261	pH 9.2 NaOH	pH 9.2 NaOH	1.0 mM
Salicylic acid	297	pH 3.0 HCl	pH 3.0 HCl	1.0 mM

(iii) R_M describes the barrier to diffusion through the lipid impregnated filter.

Eqns. 2 and 3 show that $\vec{R}_T$ is linearly dependent upon $W^{-1/2}$. Thus, a plot of $\vec{R}_T$ versus $W^{-1/2}$ has an intercept defined by Eqn. 4.

$$\text{Intercept} = R'_T = 2R_I + R_M \quad (4)$$

R'_T is the intrinsic total resistance of the membrane since, at infinite rotation speed, the stagnant layers are eliminated.

In vitro skin diffusion cell studies

To determine the accuracy with which the IPM membrane predicted permeation through human skin, data were obtained for the resistance of excised human cadaver skin to the model penetrants. The diffusion cell used was of standard construction (Franz, 1975) and consisted of two compartments between which the skin sample was clamped. The receptor compartment was filled with approximately 13 ml (accurately measured) freshly prepared degassed isosmotic pH 7.4 phosphate-buffered saline containing 0.001% v/v phenylmercuric nitrate as preservative; the receiver solution was continuously stirred with a small magnetic bar. Because the low aqueous solubility of hydrocortisone may have limited the rate of removal from the dermal side of the skin, 50% v/v ethanol was used as the receptor medium for this compound (Barry, 1983).

Strips of full thickness human skin (ca. 4 cm by 15 cm) were obtained post mortem (within 24 h of death) from the abdominal region. Skin was predominately taken from female donors of caucasian origin. However, male donors were occasionally used, providing skin hair density was low. Donor age varied between 70 and 85 years. Immediately following removal of the skin from the cadaver, the subcutaneous fat was removed with a scalpel held flat over the sample, taking care not to cut into the dermis. The skin samples obtained were either used immediately or stored flat in sealed evacuated plastic bags at 4°C overnight.

The skin sample was cut to the appropriate size and mounted dermal side downwards between the two ground glass faces of the cell. The receptor compartment was maintained at 37°C in a thermostated water bath, the donor was exposed to ambient laboratory temperature (~ 20°C). The temperature of the skin surface under these conditions was 30(±2)°C as determined with a thermocouple probe. The skin and receptor solution were equilibrated for 1 h and any air bubbles that had formed under the dermis were removed by gently rocking the cell. After equilibration the penetrant was applied as a 2 ml aqueous solution of similar composition and concentration to that used in the RDC experiments. 0.5-ml samples were removed from the receptor at regular time intervals and analysed for solute by HPLC with a UV detector set at the appropriate wavelength (Table 1). Each sample was replaced with an equivalent volume of buffer prethermostated to 37°C and the receptor concentration was corrected for dilution. Experiments were conducted in at least triplicate.

Total skin resistances (R_S) were calculated from the mean steady-state fluxes (J) according to Eqn. 5.

$$R_S = \Delta C_s / J \quad (5)$$

where ΔC_s is the concentration gradient across the skin membrane.

HPLC analysis

For all compounds except NI, the system used was similar to that employed by Hadgraft et al., 1986. A Techopak, 10 μm particle size, C18 reverse-phase chromatographic column (250 mm × 4 mm) and a guard column packed with Co : Pell ODS (30–38 μm particle size) were connected to the HPLC system. A 100 μl S.G.E. all-glass syringe was used to inject excess sample into a 50-μl loop.

For NI, the HPLC system consisted of a Kontron LC T-414 reciprocating pump and pressure gauge, a fixed-volume Rheodyne sample injector with a 100-μl loop and a Uvikon LC 720 variable-wavelength UV monitor. A Spectra-Physics SP4270 computing integrator and chart recorder was connected to the UV monitor. A Spherisorb ODS I, 5 μm particle size, C18 reverse-phase chromatographic column (250 mm × 4 mm) and a guard column packed with Co : Pell ODS were connected to the HPLC system.

All assays were performed at ambient tempera-

TABLE 2

HPLC mobile phases and conditions for the analyses of the model penetrants

Compound	Mobile phase	Flow rate (ml/min)	Retention time (min)
Amylobarbitone	methanol 500 ml pH 7.4 buffer 500 ml	1.7	6.0
Barbitone	methanol 400 ml pH 7.4 buffer	1.2	4.1
Butobarbitone	methanol 500 ml pH 7.4 buffer 500 ml	1.2	6.5
Phenobarbitone	methanol 500 ml pH 7.4 buffer 500 ml	1.2	4.3
Hydrocortisone	methanol 700 ml distilled water 300 ml	1.5	4.2
Isoquinoline	methanol 700 ml distilled water 300 ml	1.4	4.0
Nicotine	methanol 500 ml acetonitrile 200 ml pH 5.2 buffer 200 ml	2.2	4.3
Salicylic acid	methanol 500 ml glacial acetic acid 12.5 ml distilled water 500 ml	1.9	4.2

ture. The columns were allowed to equilibrate for 1 h before use and were flushed with methanol-distilled water (70 : 30) at the end of each day. Column pressure was maintained between 1500 and 2500 psi.

The individual chromatographic conditions for each solute are given in Table 2.

Results and Discussion

RDC experiments

Fig. 1 shows typical plots of $\vec{R}_T$ obtained at the 4 rotation speeds for 3 of the barbiturates studied. Each point represents the mean ± S.E.M. of 6 determinations and the line drawn through the data represents the line of best fit as determined

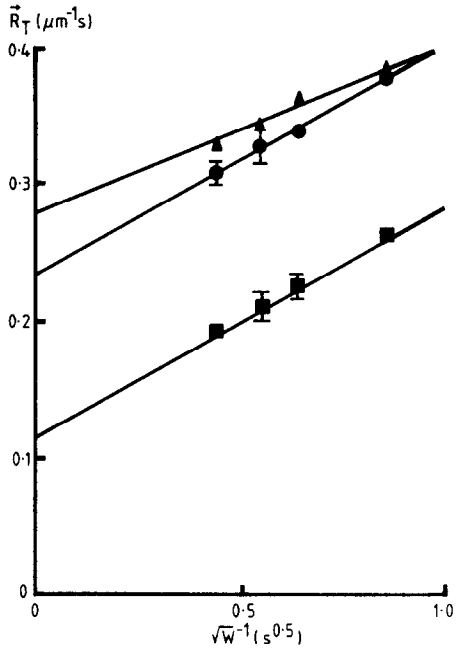

Fig. 1. Plots of $\vec{R}_T$ as a function of rotation speed for the transport of barbiturates across an IPM impregnated membrane in the RDC at 32 °C. ■, Amylobarbitone; ●, butobarbitone; ▲, phenobarbitone.

by linear regression analysis. In all cases the correlation coefficient (r) was greater than 0.99. Table 3 gives the values of R'_T obtained for each of the model penetrants. R'_T for salicylic acid was in agreement with a previously published value (Guy and Hadgraft, 1981) and was shown to be independent of donor concentration.

To examine the influence that the physicochemical nature of the penetrant exerts on R'_T a correlation analysis was performed. Correlations of the reactivities of organic compounds are usually linear relationships involving logarithms of rate or equilibrium constants (Shorter, 1973). The IPM–water partition coefficient (K_i) of a substance is an equilibrium constant and R'_T is an inverse rate constant. Thus, if there is a structure-activity relationship for permeation through the IPM membrane, a plot of log R'_T vs log K_i should be linear. Values of K_i were determined for each compound at 32 °C using the filter-probe technique (Tomlinson, 1982) and are given in Table 3. Fig. 2 shows the relationship between these

TABLE 3

Values of R'_T obtained for the transport of the model compounds across an IPM impregnated filter in the RDC and the values of K_i at 32 °C

Compound	R'_T* ($\mu m^{-1}s$)	Log R'_T	K_i *	Log K_i
Barbitone	1.828 ± 0.047	6.26	0.30 ± 0.01	−0.54
Hydrocortisone	1.220 ± 0.004	6.09	0.59 ± 0.04	−0.20
Phenobarbitone	0.277 ± 0.003	5.44	2.09 ± 0.04	0.32
Butobarbitone	0.235 ± 0.001	5.37	3.13 ± 0.02	0.50
Amylobarbitone	0.116 ± 0.001	5.06	9.42 ± 0.21	0.97
Nicotine	0.241 ± 0.006	5.38	2.01 ± 0.01	0.30
Salicylic acid	0.036 ± 0.001	4.56	27.04 ± 0.30	1.43
Isoquinoline	0.030 ± 0.003	4.48	27.84 ± 0.31	1.44

* Values are means ± S.E.M.

parameters for the penetrants studied and shows that R'_T decreases as K_i increases. Regression analysis yielded the best fitting line:

$$\log K_i = -0.879 \log R'_T + 5.79 \quad r = -0.989$$

Houk and Guy (1987) have demonstrated a similar relationship up to log $K_i \sim 1$ for series of phenols and nicotinates using the RDC. However, further increase in K_i above this point led to an increase in R'_T. They interpret this as indicating that, for lipophilic substances, interfacial transfer becomes the rate-limiting step in the overall permeation process. With respect to our data, K_i was not sufficiently high for this effect to be observed.

In vitro cadaver skin diffusion experiments

Fig. 3 shows a typical profile of the amount of PH (M) permeating the skin membrane as a function of time. Steady-state flux (J) was determined from the linear portion of the profile. Table 4 gives the values of R_S obtained for each of the model penetrants. A similar correlation analysis to that with the IPM data was performed.

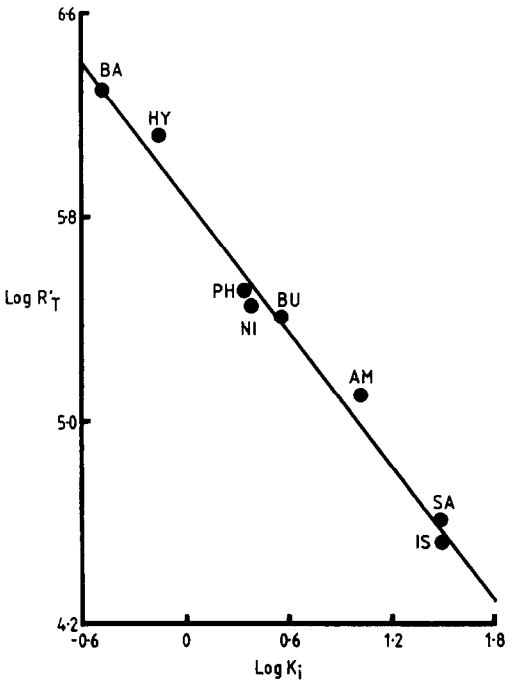

Fig. 2. Plot of log R'_T versus log K_i for transport of the model compounds across an IPM-impregnated membrane in the RDC at 32 °C.

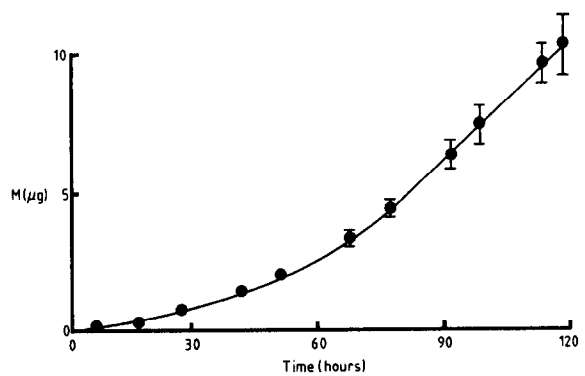

Fig. 3. Profile of the amount (M) of phenobarbitone permeating excised human skin from a 2.5 mM aqueous solution as a function of time.

TABLE 4

Values of R_S for the permeation of the model compounds through excised human skin and the values of K_o at 32 °C

Compound	R_S* ($\mu m^{-1}s$)	Log R_S	Log K_o
Barbitone	3225.8 ± 520.3	9.51	0.65
Nicotine	18.6 ± 5.0	7.27	1.17
Phenobarbitone	793.7 ± 56.7	8.90	1.47
Hydrocortisone	3030.3 ± 275.5	9.48	1.53
Butobarbitone	1851.9 ± 137.2	9.27	1.65
Amylobarbitone	158.7 ± 11.1	8.20	1.95
Isoquinoline	21.5 ± 1.7	7.33	2.03
Salicylic acid	57.5 ± 4.7	7.76	2.26

* Values are means ± S.E.M.

As stratum corneum–vehicle partition coefficients are difficult to measure, octanol–water buffer partition coefficients (K_o) were employed (Hansch and Leo, 1981). The values of K_o were taken from the data of Hansch and Anderson (1967) and Leo et al. (1971) and are given in Table 4. Fig. 4 shows the resulting relationship; as with the IPM membrane, a generally inverse linear relationship was found. However, the skin showed a much reduced resistance to isoquinoline and nicotine. The line drawn through the remaining points represents the best fit as described by linear regression analysis:

$$\log R_S = -1.08 \log K_o + 10.37 \quad r = -0.813$$

The correlation coefficient is poor; this may be due to the rank order of K_o values being different to K_{sc}, to an inadequate number of data points or to the high variability associated with biological systems. The structures of IS and NI contain a nitrogen within a carbon ring, similar to those of known penetration enhancers, such as 1-methyl-2-pyrrolidone and 1-dodecylazacycloheptan-2-one (Azone). Thus, these compounds may have some penetration enhancer activity and promote their own absorption.

Comparison between IPM and skin resistances

The accuracy with which the IPM-impregnated membrane predicts percutaneous absorption in human skin may be determined by comparing the resistances obtained for each compound by each method. Fig. 5 shows that a linear relationship

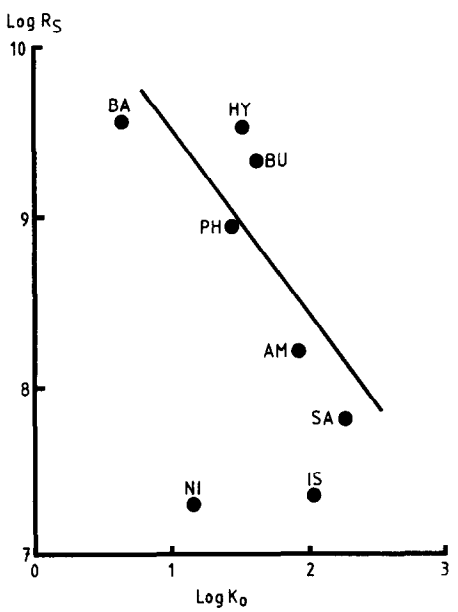

Fig. 4. Plot of log R_S vs log K_o for permeation of the model compounds through excised human skin from aqueous solution.

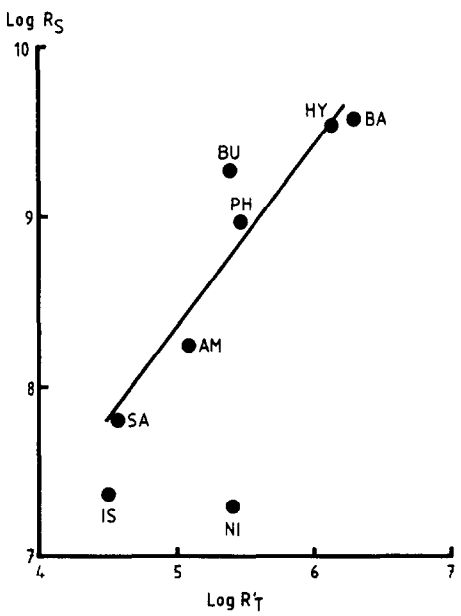

Fig. 5. Plot of log R_S for permeation of the model compounds through excised human skin from aqueous solution vs log R'_T for transport across an IPM-impregnated membrane in the RDC.

was found between log R'_T and log R_S, with the exception of IS and NI. The line drawn through the remaining points represents the best fit as described by linear regression analysis:

$$\log R_S = 1.06 \log R'_T + 3.09 \quad r = 0.925$$

The slope (1.06) indicates that the two resistances are related 1 : 1, although the barrier of excised skin is 1000-fold greater in magnitude than the artificial membrane. This may in part be accounted for by volume differences between the transport pathways through the stratum corneum compared to that through the model lipid barrier. There is increasing evidence that the major route of drug diffusion through the stratum corneum is via tortuous pathways traversing the intercellular lipids which account for ~ 5–30% of the stratum corneum volume (Elias, 1981). In contrast, the IPM membrane is a homogeneous structure in which the path length may be assumed to be the thickness of the filter support.

Thus, it may be concluded that the IPM membrane represents a reasonable model of the barrier properties of the stratum corneum. However, it fails to predict the relatively high permeability of the skin to isoquinoline and nicotine. The rotating diffusion cell model could be further improved by the use of an alternative lipid or lipids more representative of those naturally occurring within the stratum corneum (Elias et al., 1981).

Acknowledgements

We thank SERC for an award to GR, Glaxo Group Research for a gift of hydrocortisone and Professor R.H. Guy for helpful comment.

References

Albery, W.J., Burke, J.F., Leffler, E.B. and Hadgraft, J., Interfacial transfer studied with a rotating diffusion cell. *J.C.S. Faraday Trans. I,* 72 (1976) 1618–1626.

Albery, W.J. and Hadgraft, J., Percutaneous absorption: in vivo experiments. *J. Pharm. Pharmacol.*, 31 (1979) 140–147.

Barry, B.W., Dermatological formulations: percutaneous absorption. In J. Swarbrick, (Ed.), *Drugs and The Pharmaceutical Sciences, Vol. 18,* Dekker, New York, pp. 127–233.

Bronaugh, R.L. and Maibach, H.I. (Eds.), Percutaneous absorption. In *Dermatology, Vol. 6,* Dekker, New York, 1985, pp. 3–242.

Elias, P.M., Lipids and the epidermal permeability barrier. *Arch. Derm. Res.,* 270 (1981) 95–117.

Elias, P.M., Cooper, E.R., Korc, A. and Brown, B.E., Percutaneous transport in relation to stratum corneum structure and lipid composition, *J. Invest. Dermatol.,* 76 (1981) 297–301.

Feldmann, R.J. and Maibach, H.I., Regional variation in percutaneous penetration of ^{14}C cortisol in man. *J. Invest. Dermatol.,* 48 (1967) 181–183.

Flynn, G.L., Bronaugh, R.L. and Maibach, H.I. (Eds.), Percutaneous absorption, In *Dermatology, Vol. 6,* Dekker, New York, 1985, pp. 17–42.

Franz, T.J., Percutaneous absorption. On the relevance of in vitro data. *J. Invest. Dermatol.,* 64 (1975) 190–195.

Guy, R.H. and Hadgraft, J., Interfacial transport of salicylic acid. *J. Coll. Int. Sci.,* 81 (1981) 69–74.

Hadgraft, J., Walters, K.A. and Wotton, P.K., Facilitated percutaneous absorption: a comparison and evaluation of two in vitro models. *Int. J. Pharm.,* 32 (1986) 257–263.

Hansch, C. and Anderson, S.M., The structure-activity relationship in barbiturates and its similarity to that in narcotics. *J. Med. Chem.,* 10 (1967) 745–753.

Hansch, C. and Leo, A. *Substituent Constants for Correlation Analysis in Chemistry and Biology,* Wiley-Interscience, New York, 1981.

Houk, J. and Guy, R.H., Membrane models for skin penetration studies. *Chem. Rev.,* in press.

Leo, A., Hansch, C. and Elkins, D., Partition coefficients and their uses. *Chem. Rev.,* 71 (1971) 525–616.

Maibach, H.I. and Lowe, N.J. (Eds.) *Models in Dermatology, Vol. 2. Dermatopharmacology and Toxicology.* Karger, Basel, 1985, pp. 159–188.

Poulsen, B.J., Young, E., Coquilla, V. and Katz, M., Effect of topical vehicle composition on the in vitro release of fluocinolone acetonide and its acetate ester. *J. Pharm. Sci.,* 57 (1968) 928–933.

Scheuplein, R.J., Mechanism of percutaneous absorption I. Routes of penetration and the influence of solubility, J. Invest. Dermatol., 45 (1965) 334–346.

Shorter, J., *Correlation Analysis in Organic Chemistry: an Introduction to Linear Free Energy Relationships.* Clarendon, Oxford, 1973, pp. 1–7.

Tomlinson, E., Filter-probe extractor: a tool for the rapid determination of oil-water partition coefficients. *J. Pharm. Sci.,* 71 (1982) 602–604.