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Development of model membranes for percutaneous absorption measurements. II. Dipalmitoyl phosphatidylcholine, linoleic acid and tetradecane

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Summary

The use of the rotating diffusion cell and associated lipid membrane as an in vitro model for percutaneous absorption has been documented. In a previous report, the transport resistances of an isopropyl myristate membrane to 8 model penetrants were contrasted with those for excised human cadaver skin. The results indicated that, although some degree of correlation between the two was evident, the predictability of the model system could be improved. Three alternative lipid models for the stratum corneum (the epidermal penetration barrier) have been investigated. These were dipalmitoyl phosphatidylcholine, linoleic acid and tetradecane. The transport resistances of each of the artificial lipid membranes to each of the model penetrants was determined and the correlations with human skin were reassessed. For the penetrants studied, the tetradecane membrane appeared to offer the best correlation with human skin. The transport resistance provided by this membrane was only 100-fold less than that of the human skin in vitro. This represents an order of magnitude improvement in predictability over that of the previously studied isopropyl myristate membrane. The rotating diffusion cell with a tetradecane membrane warrants further investigation as a predictive model for percutaneous absorption.

Introduction

A reliable in vitro model for absorption across human skin would permit the screening of drug candidates for transdermal delivery and allow the prediction of adverse reactions arising from dermal exposure to industrial and environmental toxinogens. Such a system would alleviate the need for expensive, time-consuming and potentially hazardous in vivo approaches using animals or humans, and remove biological variability.

It is generally accepted that the outermost skin layer, the stratum corneum, is the rate-limiting barrier in the transport of most chemicals across the skin (Scheuplein, 1965). Thus, an in vitro model for skin penetration should successfully mimic the barrier properties of human stratum corneum. The utility of the rotating diffusion cell

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(RDC) as a system in which to set up such an in vitro model has been studied previously (Albery and Hadgraft, 1979a; Guy and Fleming, 1979; Houk and Guy, 1987). Most recently, the RDC was used to measure the transport parameters of a diverse group of 8 model penetrants across an artificial lipid membrane (Hadgraft and Ridout, 1987). The membrane consisted of a membrane filter impregnated with isopropyl myristate (IPM), a liquid that has been extensively employed as a model for skin lipids (Poulsen et al., 1968; Albery and Hadgraft, 1979b). The transport resistance of the IPM membrane to each of the penetrants was compared with the corresponding barrier to permeation through excised human cadaver skin. A reasonable correlation existed between the two parameters. However, the model membrane was unable to predict the relatively low transport resistance offered by the skin to isoquinoline and nicotine. Overall, the skin offered a resistance to penetration that was 1000-fold greater than IPM.

The objective of the work described in this paper was to improve the predictability of the in vitro system using alternative lipids to form the artificial membrane barrier. The predominant lipids present in the intercellular spaces of human stratum corneum are ceramides, free sterols, free fatty acids, with small proportions of glycolipids, triglycerides, sterol esters, cholesterol sulphate and hydrocarbons (Elias, 1983; Downing et al., 1987). Guy and Fleming (1979) used the RDC to estimate the diffusion coefficients of methyl and ethyl nicotinates in a series of organic membranes. The lipids studied included dipalmitoyl phosphatidylcholine (DPPC), linoleic (octadecadienoic) acid (LA), IPM and tetradecane (TD). IPM mimics, in a simplistic fashion, the amphiphilic nature of naturally occurring lipids. Linoleic acid (LA) is important in the formation of stratum corneum barrier function (Elias et al., 1980; Houtsmuller and Van der Beek, 1981). The use of DPPC as a stratum corneum model, both singularly and in combination with other lipids, has been reported previously (Firestone and Guy, 1985; Guy and Fleming, 1979; Scheuplein and Ross, 1974). TD was selected to represent the non-polar *n*-alkanes that are found in the stratum corneum lipid matrix. Guy and Fleming (1979) concluded that all 3 lipids might offer reasonable models for the epidermal barrier. It was decided, therefore, to investigate further the use of these lipids in the RDC as alternatives to IPM.

The model penetrants were the same as those employed previously: salicylic acid (SA), isoquinoline (IS), nicotine (NI), hydrocortisone (HY), and 4 barbiturates (amylobarbitone (AM), barbitone (BA), butobarbitone (BU) and phenobarbitone (PH)). The transport of these chemicals across each of the model lipid membranes was determined and the resulting resistance data were compared with the corresponding values for excised human skin.

Materials and Methods

Materials

AM, PH (both May and Baker); BA (Boots Company); BU (Sigma Chemical Company); 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. 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

The RDC methodology has been described previously (Hadgraft and Ridout, 1987). The model membrane consists of a 0.2-μm cellulose nitrate membrane filter saturated with the chosen lipid. Permeation of solute was followed from an aqueous donor solution through the artificial membrane into an aqueous receptor phase. To minimise effects due to solute ionisation, the pH of the donor and receptor phases was maintained constant. The actual composition of the donor and receptor phases used for each penetrant and the initial donor concentrations are given in Table 1.

In the RDC, stagnant diffusion layers are created on either side of the membrane. The thickness (Z_D) of these layers is related to the rotation

TABLE 1

The composition of the donor and receptor phases used in RDC experiments

Compound	Donor phase	Receptor phase	Donor concentration
AM	pH 5.0 NaOH	pH 7.4 buffer	2.5 mM
BA	pH 5.0 NaOH	pH 7.4 buffer	10.0 mM
BU	pH 5.0 NaOH	pH 7.4 buffer	2.5 mM
PH	pH 5.0 NaOH	pH 7.4 buffer	2.5 mM
HY	Ethanol : water 5 : 95, v/v	pH 7.4 buffer	1.0 mM
IS	pH 7.4 NaOH	pH 7.4 NaOH	1.0 mM
NI	pH 9.2 NaOH	pH 9.2 NaOH	1.0 mM
SA	pH 3.0 HCl	pH 3.0 HCl	1.0 mM

speed (W). Reciprocal forward rate constants (resistance, $\vec{R}_T$) are plotted against $W^{-1/2}$ and extrapolation of the resultant linear relationship to infinite rotation speed (where Z_D is effectively zero) yields the intrinsic total membrane resistance to solute permeation (R'_T). The mean values of R'_T for each penetrant crossing each lipid membrane were determined using at least 5 different membranes.

Results

Linoleic acid

(a) RDC theory predicts that the slope of the $\vec{R}_T$ vs $W^{-1/2}$ plot is independent of the lipid membrane when donor and receptor phases are aqueous (Albery et al., 1976). To confirm this hypothesis, $\vec{R}_T$ was determined as a function of W for HY, IS and SA permeating a LA membrane. Fig. 1 compares the LA data obtained for SA with those measured previously using an IPM membrane. Each point represents the mean ± S.E.M. of 6 determinations and the line drawn through the IPM data represents the best fit as determined by linear regression analysis. The slope obtained using the IPM membrane is drawn through the LA data. For all penetrants, the values of the slopes of the $\vec{R}_T$ vs $W^{-1/2}$ plots were independent of membrane type (Table 2); confirming, once again, the validity of the RDC technique.

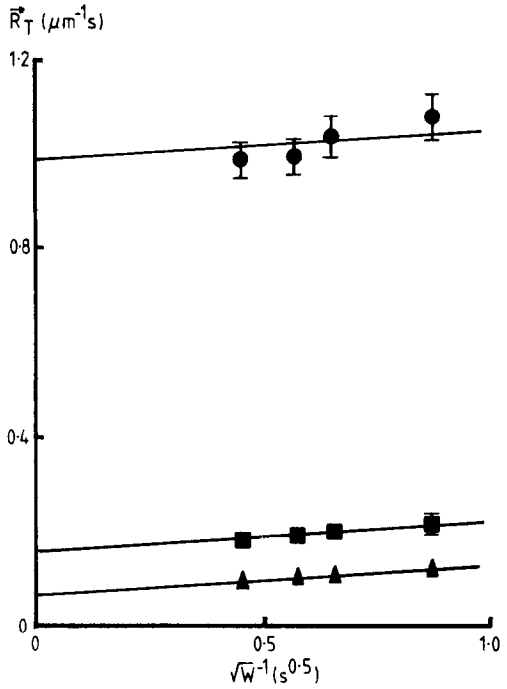

Fig. 1. Plot of $\vec{R}_T$ as a function of rotation speed for the transport of SA across different lipid-impregnated membranes in the RDC at 32 °C. ●, DPPC; ■, LA; ▲, IPM.

LA was found to be sufficiently soluble in pH 7.4 phosphate buffer to be washed off the filter when the buffer was used as the receptor phase. To prevent this, pH 7.0 sodium hydroxide was used to replace the buffer solution. The receptor solution pH was continuously monitored and small quantities of 0.1 mM sodium hydroxide solution

TABLE 2

Slopes of the $\vec{R}_T$ vs $W^{-1/2}$ plots using IPM and LA membrane at 32 °C

Compound	Slope ($\mu m^{-1}\ s^{1/2}$)	
	IPM	LA
AM	0.166 ± 0.004	–
BU	0.175 ± 0.011	–
PH	0.125 ± 0.007	–
HY	0.302 ± 0.011	0.333 ± 0.030
IS	0.220 ± 0.007	0.221 ± 0.004
NI	0.130 ± 0.008	–
SA	0.100 ± 0.001	0.113 ± 0.005

Values are mean ± S.E.M.

TABLE 3

Values of R'_T obtained for the transport of the model compounds across either LA- or DPPC-impregnated membranes in the RDC at 32 °C and the values of K_o

Compound	R'_T ($\mu m^{-1}s$)		Log K_o
	LA	DPPC	
AM	0.323 ± 0.001	3.76 ± 0.12	1.95
BA	1.540 ± 0.210	11.06 ± 0.45	0.65
BU	0.341 ± 0.006	–	1.65
PH	0.608 ± 0.009	–	1.47
HY	1.090 ± 0.011	6.58 ± 0.13	1.53
IS	0.007 ± 0.007	2.32 ± 0.15	2.03
NI	0.207 ± 0.032	5.94 ± 0.10	1.17
SA	0.132 ± 0.001	0.93 ± 0.06	2.26

Values are mean ± S.E.M.

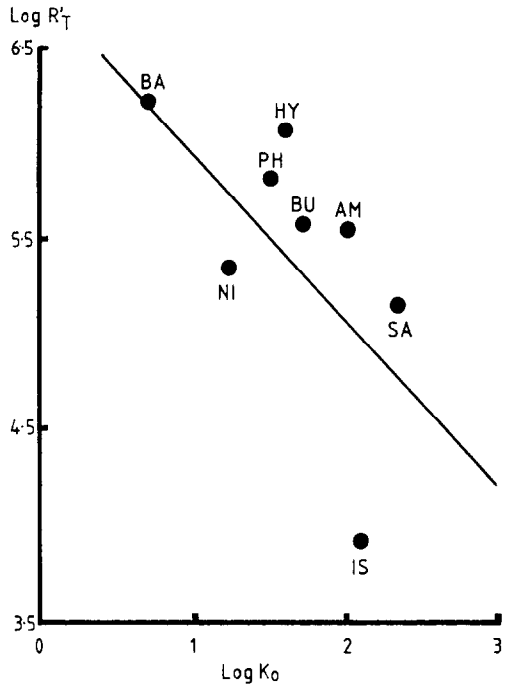

Fig. 2. Plot of log R'_T versus log K_o for the transport of the model compounds across a LA-impregnated membrane in the RDC at 32 °C.

added to maintain pH 7.0. To demonstrate that LA did not wash off the filter at pH 7.0, the transport resistance to SA was determined using a membrane that had been previously exposed to a solution of pH 7.0 sodium hydroxide for 5 h. The resistance of the membrane to SA was not significantly different from that obtained previously.

R_T for the barbiturates and nicotine were measured at $W = 5$ Hz and the respective values of R'_T were evaluated by extrapolation using the slopes obtained previously with IPM (Table 2) (Hadgraft and Ridout, 1987). For barbitone, where resistance was high, the value of R_T obtained at 5 Hz was assumed to be equivalent to R'_T. The values of R'_T for each compound are given in Table 3.

To examine the influence of penetrant physicochemical properties on R'_T, a correlation analysis with the octanol–aqueous partition coefficient (K_o), the traditionally used index of lipophilicity, was performed. The values of K_o were from Hansch and Anderson (1967) and Leo et al. (1971), and are given in Table 3. Fig. 2 shows the resulting relationship between log R'_T and log K_o; as with the IPM membrane, a generally inverse linear relationship was apparent:

$$\log R'_T = -0.87 \log K_o + 6.80 \quad r = -0.618$$

The correlation is not as good as that demonstrated for IPM and may indicate the limitations of using K_o. The LA membrane showed a reduced resistance to IS (by a factor of more than 10) than would be predicted by K_o. However, we note that for the analogous series of barbiturates alone, where the rank order of K_o should parallel that of the LA–aqueous partition coefficient, the correlation significantly improves, $r = -0.975$.

(b) The degree to which the LA model membrane can predict percutaneous absorption across human skin was determined by comparing the values of R'_T with those obtained previously for excised human skin (R_S) (Hadgraft and Ridout, 1987). Fig. 3 shows that the relationship is essentially linear; regression analysis yielded the best fitting line:

$$\log R_S = 1.03 \log R'_T + 2.90 \quad r = 0.786$$

Although the two resistances are directly related, the correlation with the earlier IPM data was more convincing. However, LA did predict the higher

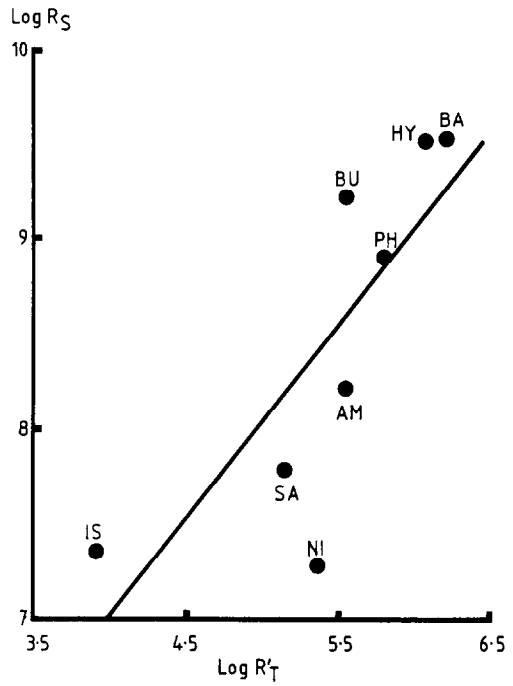

Fig. 3. Plot of log R_S for permeation of the model compounds through excised human skin vs log R'_T for transport across a LA-impregnated membrane in the RDC.

permeability of IS, which IPM did not. Finally, as for IPM, R'_T is, in general, 1000-fold less than R_S.

Dipalmitoyl phosphatidylcholine

(a) Firestone and Guy (1985) described a modified membrane impregnation technique to ensure even loading of the filter with phospholipid. This technique was adopted in the present study and is as follows. The filter was immersed in a 1% w/v solution of DPPC in chloroform, removed to allow the chloroform to evaporate and then reimmersed in the lipid solution. This process was repeated 6 times and then a second non-impregnated 0.2 μm filter was used to cover the lower surface of the lipid membrane to prevent DPPC from being thrown off into the outer aqueous compartment by cell rotation.

Resistance data at 4 rotation speeds were obtained for IS and SA. Fig. 1 shows the data for SA in comparison with those obtained using IPM and LA. Again, the IPM slopes (Table 2) were forced through the data to obtain the intercept R'_T. As before, the transport of the remaining compounds across the DPPC membrane was determined at 5 Hz only and the values of R'_T were obtained by extrapolation of the IPM slopes through this point (Table 3).

Correlation analysis was performed between membrane resistance and K_o. Fig. 4 shows that R'_T again decreases with increasing K_o. Regression analysis yielded the best fitting line:

$$\log R'_T = -0.56 \log K_o + 7.49 \quad r = -0.894$$

The slope was significantly lower than that obtained for the lipids used previously, an indication, perhaps, that this membrane is relatively insensitive to penetrant lipophilicity over the narrow range studied. Firestone and Guy (1985) reported a similar finding when the transport of nicotinates and phenols was studied through a mixed lipid membrane. The magnitude of the resistances reported here, using DPPC membranes,

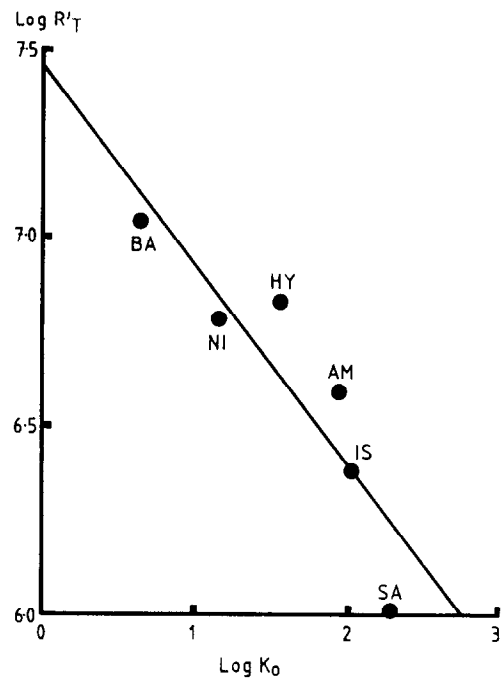

Fig. 4. Plot of log R'_T vs log K_o for the transport of the model compounds across a DPPC-impregnated membrane in the RDC at 32° C.

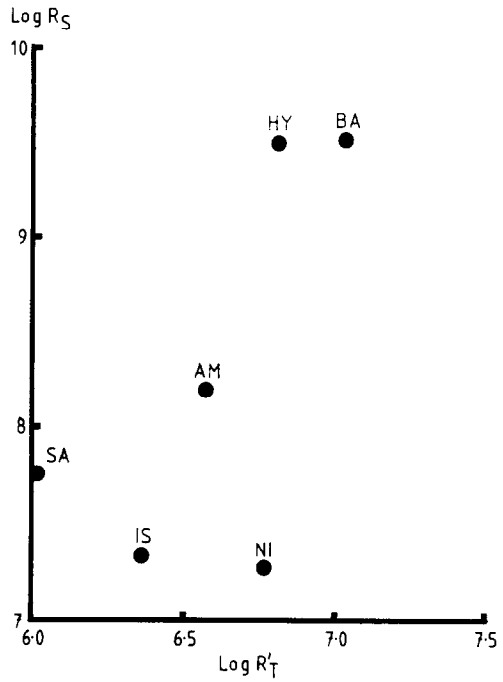

Fig. 5. Plot of log R_S for permeation of the model compounds through excised human skin vs log R'_T for transport across a DPPC-impregnated membrane in the RDC.

was comparable to the earlier results obtained with the more complex lipid mixture.

(b) Fig. 5 shows the relationship between DPPC membrane and excised skin resistances; the higher permeabilities of IS and NI through the skin were not predicted by the DPPC membrane. There is an overall trend of increasing R_S with increase in R'_T, but the scatter is insufficient to justify a linear regression analysis.

(c) As the stratum corneum lipid matrix is largely devoid of phospholipids (Elias, 1981), lipids more representative of those thought to be present in human stratum corneum may provide an improved model membrane. Thus, a mixture of sphingomyelin, cerebrosides and sulphatides (brain extract type VIII, from bovine brain) was used to form the membrane. The presence of these 3 classes of lipid in human stratum corneum has been demonstrated by Elias (1981). However, the transport characteristics of BA, IS and SA across a membrane impregnated with this lipid mixture were similar to those for the DPPC membrane.

TABLE 4

Values of R'_T obtained for the transport of the model compounds across TD impregnated membranes in the RDC and the values of K_t at 32 °C

Compound	R'_T ($\mu m^{-1}s$)	K_t	Log K_t
BA	31.85 ± 0.71	0.0022 ± 0.0001	−2.66
PH	19.84 ± 0.34	0.0038 ± 0.0001	−2.42
HY	27.81 ± 2.79	0.0079 ± 0.0002	−2.10
BU	5.02 ± 0.21	0.0096 ± 0.0003	−2.02
AM	2.93 ± 0.15	0.046 ± 0.001	−1.34
SA	1.12 ± 0.03	0.050 ± 0.001	−1.30
NI	0.27 ± 0.02	1.16 ± 0.03	0.06
IS	0.03 ± 0.01	10.68 ± 0.21	1.03

Values are mean ± S.E.M.

Tetradecane

(a) The TD membrane provided, in general, a 10-fold greater resistance to transport than IPM or LA. R_T was determined at 5 Hz only and R'_T was obtained by extrapolation to infinite rotation speed ($W^{-1/2} = 0$) using the IPM slopes given in

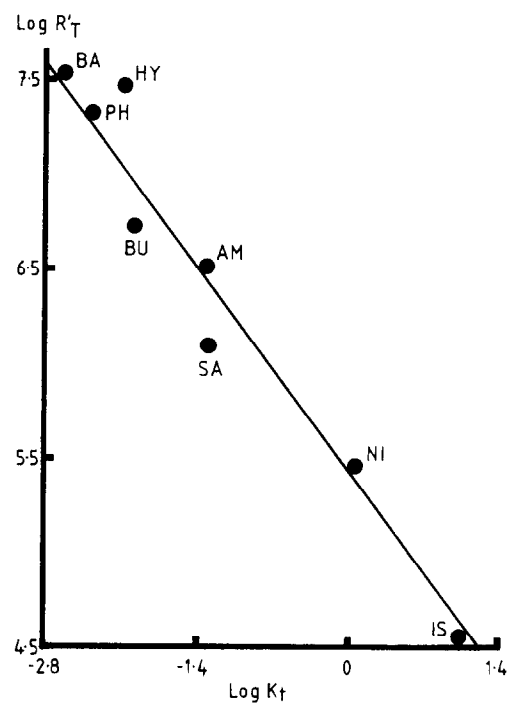

Fig. 6. Plot of log R'_T vs log K_t for the transport of the model compounds across a TD-impregnated membrane in the RDC at 32 °C.

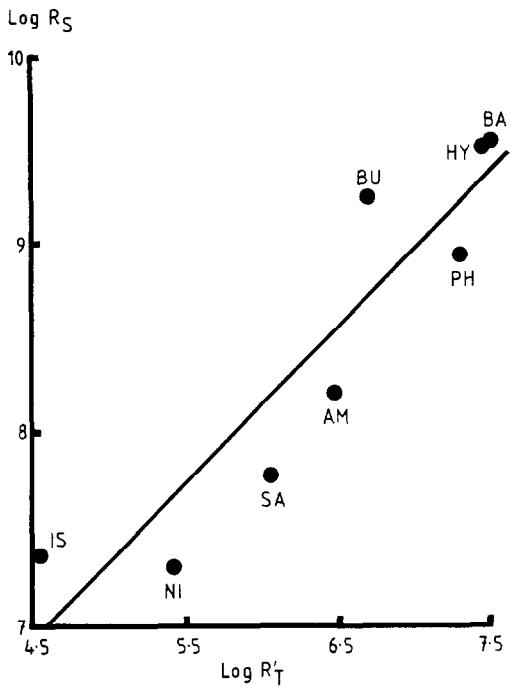

Fig. 7. Plot of log R_S for permeation of the model compounds through excised human skin vs log R'_T for transport across a TD-impregnated membrane in the RDC.

Table 2. The individual values of R'_T obtained for each compound are given Table 4. These results were correlated with the corresponding tetradecane-aqueous partition coefficients (K_t) determined using the filter-probe technique (Tomlinson, 1982). The K_t values are also given in Table 4. Fig. 6 shows the inverse linear relationship between log R'_T and log K_t. Linear regression analysis of the data gave:

$$\log R'_T = -0.83 \log K_t + 5.35 \quad r = -0.970$$

The slope, intercept and correlation coefficient were similar to those for the IPM membrane.

(b) A comparison between R'_T for the TD membrane and R_S (Fig. 7) showed that the two parameters were linearly related:

$$\log R_S = 0.82 \log R'_T + 3.22 \quad r = 0.912$$

The TD membrane predicts the low resistance of the skin to IS and NI.

The individual values of R'_T are 100-fold less than the corresponding R_S. This difference may be explained, in part, by the lower volume of lipid present in the intercellular region of the stratum corneum as compared to that in the lipid-saturated artificial membrane. The porosity of the membranes used in the RDC is of the order of 80%. The "porosity" of stratum corneum is difficult to quantify precisely; however, a figure which is an order of magnitude less than 80% seems to us quite plausible.

Discussion

The following conclusions may be drawn from this study.

(a) Of the 3 lipids employed (for the model compounds studied), tetradecane appeared to offer the best model of the barrier properties of human stratum corneum.

(b) The reproducibility of the RDC technique has been demonstrated. However, the transport of further compounds across this membrane should be determined before firm conclusions as to its suitability can be made. In this regard, a series of phenols and nicotinates, which spanned a larger range of lipophilicity, have been tested by Houk and Guy (1987) who also concluded that tetradecane offers a good model for stratum corneum lipids.

(c) Linoleic acid predicted the increased permeability of isoquinoline through skin. However, the alkaline solubility of this lipid, and the ease with which it oxidises, precludes its use as a reliable model.

(d) The permeability of membranes prepared with more complex lipids was independent of lipid composition. The lipid barriers failed to predict the increased skin permeabilities of isoquinoline and nicotine.

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