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The percutaneous absorption of phenolic compounds: the mechanism of diffusion across the stratum corneum

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The effect of temperature on the permeation of phenolic compounds from aqueous solution through excised human skin has been examined. From a thermodynamic analysis of the data, a mechanism is postulated by which these solutes penetrate through human skin. For the more polar solutes it is suggested that the main resistance to penetration is the lipid barriers in the stratum corneum. Diffusion of these substances through the stratum corneum appears to depend on the breaking of hydrogen bonds in the desolvation of the solute during this penetration process and by the overall 'viscosity' of the stratum corneum. With non-polar solutes, the aqueous boundary layers appear to provide an additional barrier to the penetration of phenolic compounds.

Substances may be transported across the skin barrier by diffusion through the appendages, hair follicles, sweat glands and sebaceous glands, or by diffusion through the stratum corneum itself. Diffusion through the appendages may contribute significantly to the transport of small non-electrolytes through the skin in the time before the establishment of steady-state conditions, and to the steady-state diffusion of solutes with extremely small permeability coefficients e.g. steroids (Scheuplein & Blank, 1971). Phenolic compounds appear to traverse excised human epidermis primarily by diffusion through the stratum corneum (Roberts, Anderson & Swarbrick, 1977 b).

According to Scheuplein (1967) and Scheuplein & Blank (1971, 1973) the intracellular keratin matrix of stratum corneum provides the main resistance to penetration by solutes, although polar and non-polar solutes appear to diffuse through the stratum corneum by different pathways. Thus, Scheuplein (1972) has suggested that polar solutes diffuse in the polar aqueous channels of the stratum corneum whereas the less polar solutes diffuse through the lipid regions. An alternative postulate is that of Yotsuyanagi & Higuchi (1972) who have suggested a two phase series model consisting of protein-rich 'cytoplasm' and a lipoidal 'cell wall' to describe the passage of alcohols and steroids across human stratum corneum. Another possibility, suggested by the studies of Flynn & Yalkowsky (1972) on the permeation of alkyl *p*-aminobenzoates across dimethyl polysiloxane membranes, is that the stratum corneum is the rate-limiting barrier for the permeation of polar solutes whereas aqueous boundary layers are important barriers to diffusion of more lipophilic solutes. To clarify the mechanism by which phenolic and other small molecules pass across human epidermis, we have examined the effects of temperature on the rates of permeation.

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MATERIALS AND METHODS

The chemicals and epidermal membranes used and the method of studying permeation rates are all as described previously (Roberts & others, 1977 b).

RESULTS AND DISCUSSION

The temperature dependence of the permeability coefficients (k_p) of several phenolic compounds is shown in Fig. 1. Mean activation energies (E_m) for permeation calculated from such Arrhenius-type plots are listed in Table 1 together with the appropriate octanol/water partition coefficients (P). Experiments were repeated anywhere from two to five times. In no case did the range observed exceed $\pm 0{\cdot}6$ kcal mol^{-1} ($2{\cdot}5$ kJ mol^{-1}). Data for several aliphatic alcohols (from Scheuplein & Blank, 1971) are also listed in Table 1. The energies of activation of all the monohydric compounds are plotted against log P in Fig. 2. It is apparent that the activation energies for permeation through the epidermis are higher for solutes with low P values. Although the precise relation between E_m and log P is equivocal, the data strongly suggest a break in the slope at a log P value of approximately 2. This would be consistent with the relation between log k_p and log P reported previously (Roberts & others, 1977 b)

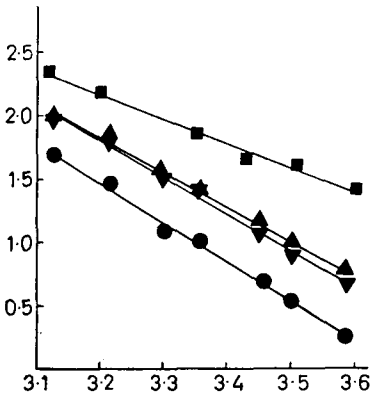

FIG. 1. Arrhenius-type plots for permeability coefficients of some phenolic compounds from aqueous solutions through human epidermis. ● phenol; ▲ *o*-cresol; ▼ *m*-cresol; ■ *p*-bromophenol. Ordinate: log ($k_p \times 10^5$ cm min^{-1}). Abscissa: $1/T \times 10^3$.

where a change in slope was also observed at a log P value of around 2.

With orthogonal polynomial curve fitting, the data are best described by the cubic equation:

$$E_m = 16{\cdot}724 + 2{\cdot}884(\log P) - 4{\cdot}175(\log P)^2 + 0{\cdot}802(\log P)^3$$

The residual sum of squares was less than with a linear model, in spite of a large contribution from the value of thymol, which appears to be an outlier. A sigmoidal rather than linear relation between E_m and log P would also be expected from theoretical considerations, since both an upper and lower limit for E_m would be predicted for the type of plot shown in Fig. 2. Thus, as log P decreases, E_m must eventually reach a finite maximum. The data for polar alcohols reported by Scheuplein & Blank (1971) and shown in Fig. 2 support such a contention. On the other hand, as log P increases, the activation energy for diffusion would be expected to fall and then level off at a minimum value, close to that for diffusion of polar non-electrolytes through liquid-filled pores. Such solution diffusion requires an activation energy of 5–7 kcal mol^{-1} (21–29 kJ mol^{-1}) (Scheuplein, 1966).

These abrupt changes in both activation energy and permeability with respect to lipophilic character may result from a change in the type of the rate-limiting barrier for permeation. To clarify the mechanism by which phenolic and other compounds traverse the stratum corneum, the diffusion behaviour of polar and non-polar solutes is examined.

Table 1. *Activation energies* (E_m) *for epidermal penetration and octanol-water partition coefficients* (P) *of phenolic compounds and alcohols.*

Solute	log P	E_m (kcal mol^{-1})
Ethanol	−0·16	16·4 (68·7)*
Propanol	0·30	16·5 (69·1)
Resorcinol	0·8	17·8 (74·5)
Butanol	0·84	16·7 (69·9)
Pentanol	1·34	16·5 (69·1)
Phenol	1·46	14·4 (60·3)
Hexanol	1·84	10·9 (45·6)
p-Cresol	1·95	13·7 (57·4)
o-Cresol	1·95	12·8 (53·6)
m-Cresol	1·96	13·6 (56·9)
m-Nitrophenol	2·00	13·3 (55·7)
o-Chlorophenol	2·15	9·6 (40·2)
Heptanol	2·34	9·9 (41·4)
p-Bromophenol	2·59	8·8 (36·8)
β-Naphthol	2·84	9·8 (41·0)
Octanol	2·84	8·7 (36·4)
Chlorocresol	3·10	10·3 (43·1)
Thymol	3·34	12·6 (52·8)
2,4,6-Trichlorophenol	3·69	9·1 (38·1)

* kJ mol^{-1}.

'Polar' solutes

A comparison of the data obtained for phenol and resorcinol indicates that the addition of a hydroxyl group increases the energy of activation for permeation by about 3 kcal mol^{-1} (12·5 kJ mol^{-1}) and

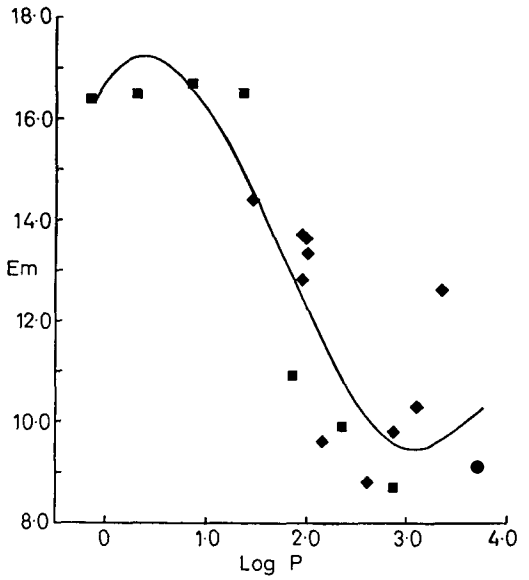

FIG. 2. Relation between the activation energy (E_m) for permeation of various solutes through human epidermis and their octanol-water partition coefficients (P). ◆ phenolic compounds; ■ aliphatic alcohols (data from Scheuplein & Blank, 1971). Ordinate: Energy of activation for permeation, E_m (kcal mol^{-1}). Abscissa: log P.

the entropy of activation by about 6 cal mol^{-1} deg^{-1} (25 J mol^{-1} deg^{-1}) with more than a 30-fold decrease in the k_p value. Scheuplein & Blank (1971) have reported similar changes for the addition of a second hydroxyl group to polar non-electrolytes. These values are consistent with the formation and rupture of two hydrogen bonds (Cohen, 1975). The incremental entropy change per hydroxyl group for the diffusion of solutes in the epidermis of 6 cal mol^{-1} deg^{-1} (25 J mol^{-1} deg^{-1}) is comparable to the entropy change for the transfer of solutes from water to a lipid phase (from the data of Cohen, 1975), but dissimilar to that for the diffusion of solutes in water where the entropy change is close to zero (from the data of Stein, 1967). Stein has suggested that for the diffusion of polyhydric solutes in water, only single hydrogen bonds need to be broken in each diffusion step. These observations are consistent with the concept of a lipid barrier phase.

Further evidence for the existence of a lipid barrier comes from the incremental changes in the activation parameters observed for non-polar groups. Scheuplein & Blank (1971) have reported similar values for the free energy, entropy and enthalpy of activation for diffusion of ethanol, 1-propanol, 1-butanol and 1-pentanol. The near-zero value for the activation entropy increment for the diffusion of the methylene group suggests a lipid barrier. By contrast, an aqueous barrier phase would be expected to involve a methylene group in a large negative entropy increment due to structuring of water around the non-polar residue.

An alternative postulate that polar solutes can enter and diffuse through the aqueous regions of the stratum corneum without passing through a lipid barrier (Scheuplein, 1972) is not supported by the diffusion behaviour of solutes via polar pathways in other epithelial membranes. Thus Wright & Pietras (1974) found that diffusion of solutes by a polar pathway was characterized by a marked dependence of the diffusion coefficient on molecular volume, low apparent energies for permeation and high permeability coefficients. Additionally, if polar pathways do exist, a reasonable osmotic water permeability may be anticipated (Wright & Diamond, 1969), particularly since the volume fraction of water in hydrated stratum corneum is reported by Scheuplein & Morgan (1967) to be greater than 0·6. However Selmanowitz & Wheatley (1968) found that wide variations in hydrostatic pressures had little effect on the permeability of the epidermis to water.

The nature and morphological composition of the lipid barrier phase cannot be readily ascertained. Solely from a consideration of the relative volumes of the intracellular keratin matrix and the thickened plasma membrane, it is probable that experimentally determined stratum corneum-water partition coefficients (K_m) are comparable to those between the intracellular keratin matrix and water (Roberts, 1976). Deductions about the nature of the stratum corneum from partition coefficient studies (Roberts, Anderson & others, 1977 a) support this postulate. The location of the main site of diffusional resistance is less readily defined. Yotsuyanagi & Higuchi (1972) contend that the 'cell wall' (thickened plasma membrane) is the main diffusional barrier whereas Scheuplein (1972) has provided evidence supporting the intracellular keratin matrix as a barrier.

It is apparent, however, from the magnitude of the diffusion coefficients of phenolic and other compounds in the human epidermis that this barrier is extremely viscous or semisolid as described by Scheuplein (1972). The experimental diffusion coefficients for phenolic compounds in human epidermis are about 10 000 times less than those for the diffusion of the same solutes in water.

'Non-polar' solutes

The unstirred boundary layers (Nernst or diffusion layers) adjacent to the membrane itself contribute to the overall diffusional resistance encountered in transport processes and must be accounted for in any diffusion model (Flynn & Yalkowsky, 1972). The general equation describing transport across a boundary layer-homogeneous lipid membrane system takes the form (Zwolinski, Eyring & Reese, 1949; Flynn & Yalkowsky, 1972)

$$\frac{1}{k_p} = \frac{2h_{aq}}{D_{aq}} + \frac{h_m}{K_m D_m} \quad .. \quad (1)$$

where k_p is the overall permeability coefficient, D_{aq} is the diffusion coefficient of the solute in the boundary layer of thickness h_{aq}, D_m is the diffusion coefficient of the solute in a membrane of thickness h_m, and K_m is the membrane-water partition coefficient. Equation (1) reduces to

$$k_p = K_m D_m / h_m \quad .. \quad .. \quad (2)$$

when diffusion in the membrane is rate-limiting and to

$$k_p = D_{aq} / 2h_{aq} \quad .. \quad .. \quad (3)$$

when diffusion in the boundary layer limits the rate.

Following the method of Zwolinski & others (1949), the reciprocals of the permeability coefficients of phenolic compounds have been plotted against

the reciprocals of their stratum corneum-water partition coefficients (Fig. 3). Equation (1) requires that such plots for a series of compounds give rise to a straight line through the origin when diffusion in the membrane itself is the rate-limiting step, whereas a horizontal line will result when diffusion in the boundary layers is limiting. Fig. 3 shows that diffusion through the aqueous boundary layers is

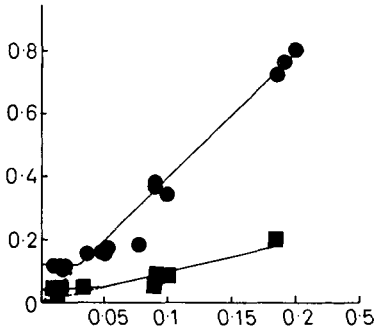

FIG. 3. Relation between k_p and K_m values of phenolic compounds as a double reciprocal plot. ● 25°; ■ 46°. Ordinate: $1/k_p \times 10^4$ (cm min^{-1}). Abscissa: $1/K_m$.

rate-limiting for solutes with large stratum corneum-water partition coefficients; at higher temperatures the boundary layer effect extends to solutes with lower partition coefficients.

This finding is not consistent with the proposition of Scheuplein (1972) that permeation of polar and non-polar solutes proceeds by diffusion along different parallel pathways. However in the experimental procedures used both by Scheuplein and in the present study the epidermal membranes have been supported by discs of porous filter paper and consequently aqueous boundary layers would be expected to be considerable. A similar effect is likely *in vivo* because of the largely aqueous composition of lower layers of skin.

Equation (1) may be modified to examine the change of k_p with temperature as follows. Stratum corneum-water partition coefficients are not significantly influenced by temperature (Anderson, Triggs & Roberts, 1976), while the dependence of diffusion coefficient on temperature can be expressed in an Arrhenius form

$$D = Ae^{-E/RT} \quad .. \quad .. \quad (4)$$

where A is the frequency factor and E is the activation energy for diffusion (Glasstone, Laidler & Eyring, 1941). Thus, combining equations (1) and (4) leads to

$$k_p = \frac{K_m A_m A_{aq}\, e^{-(E_{aq}+E_m)/RT}}{h_m A_{aq}\, e^{-E_{aq}/RT} + 2h_{aq} K_m A_m\, e^{-E_m/RT}} \quad (5)$$

where subscripts m and aq refer to the membrane and boundary layers respectively.

Fig. 4 shows Arrhenius-type plots obtained by substituting into equation (5) the typical data values shown in Table 2 along with representative values of K_m. For non-polar solutes ($K_m = 30$ or 60), a single linear relation was not found. An inflection is apparent and the slope becomes steeper below about

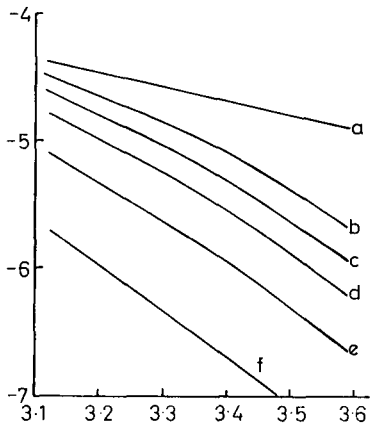

FIG. 4. Theoretical estimates of effect of boundary layers on the Arrhenius-type plots for the penetration of solutes through human epidermis (see text and Table 2 for details). a: Boundary layer alone; b: $K_m = 60$; c: $K_m = 30$; d: $K_m = 15$; e: $K_m = 5$; f: $K_m = 1$. Ordinate: log k_p (cm s^{-1}). Abscissa: $1/T \times 10^3$.

25°. At higher temperatures the effects for boundary layers are more pronounced for the non-polar solutes giving apparent energies of activation of 10 and 9 kcal mol^{-1} (42 and 37·5 kJ mol^{-1}) for $K_m = 30$ and $K_m = 60$ respectively. The inflection may be missed in permeation studies due to inadequate precision of experimental results over a limited range of temperatures. The inflection suggested by Fig. 4 is not evident in the plots for phenolic compounds (Fig. 1) although inflections in Arrhenius-type plots have been observed for alcohols by Scheuplein (1967). He attributed them to a change in fluidity of the lipids in the stratum corneum, but recent partition coefficient data do not support this contention (Roberts, Anderson & others, 1977 a). For very polar solutes, boundary layer effects are minimal and, hence, linear relations are expected (Fig. 4).

This further analysis supports the proposed role of aqueous boundary layers in the penetration of solutes

Table 2. *Representative values for stratum corneum penetration studies used in calculating the data used in Fig.* 4.

Property	Value	Reference
h_m	27 μm	Scheuplein & Blank (1973)
D_m (25°)	$0{\cdot}7 \times 10^{-9}$ cm^2 s^{-1}	Scheuplein & Blank (1973)
E_m	16·4 kcal mol^{-1} (68·7 kJ mol^{-1})	Scheuplein & Blank (1971)
E_{aq}	5·3 kcal mol^{-1} (22·2 kJ mol^{-1})	Glasstone, Laidler & Eyring (1941)
h_{aq}	200 μm	*
D_{aq} (25°)	10^{-6} cm^2 s^{-1}	†

* The appropriate boundary layer thickness is uncertain; the relatively high value of 200 μm has been chosen because the epidermis has been supported by filter-paper discs.

† A typical value which is greater than the diffusion coefficient in the membrane (D_m) because of the much lower viscosity of water.

through the epidermis *in vitro*. It is apparent that the lipophilic character of solutes and their hydrogen bonding capacity are the two main structural features determining their penetration through the epidermis. Polar solutes are retarded by a lipid barrier phase of high viscosity whereas less 'polar' solutes are retarded not only by the lipid barrier but also by contributions of the aqueous boundary layer to the total diffusional resistance.

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