# Physicochemical characterization of the human nail: permeation pattern for water and the homologous alcohols and differences with respect to the stratum corneum*

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In order to develop a basic concept of the permeability of the human nail plate and thus create a better understanding of the toxic potentials and therapeutic possibilities of substances applied to the nail, avulsed cadaver nails have been placed in specially constructed diffusion chambers and their permeation by water and the n-alkanols through dodecanol, all in high aqueous dilution, has been investigated. The permeability coefficient of water is $16.5 \times 10^{-3}$ cm $h^{-1}$ and that for methanol is $5.6 \times 10^{-3}$ cm $h^{-1}$. Ethanol's permeability coefficient measured $5.8 \times 10^{-3}$ cm $h^{-1}$. Permeability coefficients decreased systematically thereafter to a low value of $0.27 \times 10^{-3}$ cm $h^{-1}$ at n-octanol. The middle chain length alkanols, n-pentanol through n-octanol, have similar permeability coefficients but n-decanol and n-dodecanol show higher rates of permeation. The data suggest that, as a membrane, the hydrated human nail plate behaves like a hydrogel of high ionic strength to the polar and semipolar alcohols. Declining permeability rates appear linked to decreased partitioning into the complex matrix of the plate as the compounds become hydrophobic. The results for n-decanol and n-dodecanol introduce the possibility that a parallel lipid pathway exists which favours the permeation of these exceedingly hydrophobic species.

Apparently, no evidence exists concerning fundamental permeation mechanisms and possible influences of chemical structure on transport across the nail plate. To an extent its permeability properties have been inferred without foundation from the behaviour of other horny tissues. In order to make *a priori* judgements concerning toxic risk and therapeutic benefit of substances brought in contact with the nail, some baseline information on this tissue is needed.

We have shown it possible to determine nail plate permeability coefficients using standard diffusion cell techniques (Walters et al 1981). Results obtained for water agreed well with literature data on water transpiration through the nail plate (Burch & Winsor 1946; Spruit 1971; Baden et al 1973). In pursuant studies the techniques have been extended to the permeation of some n-alkanols. These are useful prototype compounds with systematically varying oil/water (o/w) distribution coefficients and diffusion

coefficients. Such structural influences on physicochemical properties, when considered together with relative permeabilities, have helped decipher the barrier mechanisms of several membranes (Blank 1964; Scheuplein 1965; Hwang et al 1976; Ho et al 1977: Behl et al 1980; Durrheim et al 1980; Flynn et al 1981). Previous studies of the alkanol's permeation of skin are especially notable as these provide evidence that the stratum corneum acts to some extent as a hydrophobic continuum (barrier) (Blank 1964; Scheuplein 1965; Behl et al 1980; Durrheim et al 1980; Flynn et al 1981). Similar studies on the human nail plate presented here are comparably revealing as, unlike the stratum corneum, the nail becomes less permeable to the n-alkanols as their hydrophobicity is increased. At extreme hydrophobicity there is increased permeability. The mechanistic significance of these general observations is considered.

## MATERIALS AND METHODS

### Materials

Tritiated water and radiolabelled alcohols were obtained from New England Nuclear ([³H]water, [³H]methanol, [¹⁴C]ethanol, [¹⁴C]butanol), California Bionuclear ([¹⁴C]propanol, [¹⁴C]pentanol,

[14C]heptanol, [14C]dodecanol) and ICN ([14C]hexanol, [14C]octanol, [14C]decanol). All radiolabelled compounds were diluted with saline (0·9% NaCl Irrigation Solution, Abbott Labs) before use. The alkanols were diluted to trace concentrations, $10^{-4}$ molar or less.

### Permeation procedures

Details of the diffusion cell and permeation procedures have been given previously (Walters et al 1981). Briefly, trimmed human nail plate sections* were placed between two halves of a diffusion cell. A known amount of a radiolabelled permeant was placed in the donor chamber and samples were taken at predetermined intervals from the receptor chamber. Isotope activity was monitored using a Beckman LS 9000 liquid scintillation counter.

The permeation behaviours of [3H]water and [3H]methanol and [14C]alkanols in dilute solution were followed as a function of time at 37 °C. In all cases two permeants were applied with different radiolabels. Generally methanol was run as a tritiated compound along with a 14C-labelled copermeant. Methanol thus served as a reference and it is important to note that the increased values for the permeability coefficient of decanol and dodecanol were obtained concurrently with normal methanol data.

Permeability coefficients (P) were calculated from:

$$P = \frac{V(dC/dt)}{A \cdot \Delta C} \qquad (1)$$

where V is the volume of the receiver half cell, dC/dt is the rate of change in concentration in the pseudo-steady state portion of the receiver concentration versus time plot, A is the diffusional area and $\Delta C$ is the concentration differential of permeant across the membrane. V(dC/dt) gives the diffusional flux in mass per unit time. The diffusion cells with nail plate membranes in place were scrupulously checked for intercompartmental leakage using soluble but impenetrable polyethyleneglycol markers and no leaks were evident.

Diffusivities of the permeants in the nail plate tissue were calculated from the non-stationary state periods using:

$$D_{eff} = \frac{h^2}{6t_L} \qquad (2)$$

Where $D_{eff}$ is the effective diffusivity for a given

* Fresh cadaver nails generously supplied by Dr T. M. Oelrich, University of Michigan, School of Medicine.

compound, h is the nail plate thickness and $t_L$ is the diffusional lag time obtained by linear regression of the steady state slope of uptake versus time plots. The nail plates used in these studies were measured with a micrometer and averaged 0·54 mm in thickness.

### RESULTS AND DISCUSSION

Permeability coefficients of water and the saline diluted n-alkanols are given along with diffusion lag times in Table 1. Fig. 1 shows the relationship between the logarithms of the permeability coefficients and the alkyl chain lengths of the alcohols. An unusual pattern is observed with minimum permeability coefficient values at intermediate alkyl chain length.

Table 1. Nail plate permeability data for water and n-alkanols.

| Permeant | Permeability[a] coefficient (cm h$^{-1}$ × 10$^3$) | Lag. time ($t_L$) (s) | Effective diffusion[b] constant ($D_{eff}$) cm$^2$ s$^{-}$ × 10$^7$ |
|---|---|---|---|
| Water | 16·5 ± 5·9 (6) | 900 ± 100 | 5·4 |
| Methanol | 5·6 ± 1·2 (26) | 1790 ± 200 | 2·7 |
| Ethanol | 5·8 ± 3·1 (8) | 2730 ± 200 | 1·3 |
| n-Propanol | 0·83 ± 0·15 (4) | 4020 ± 350 | 1·2 |
| n-Butanol | 0·61 ± 0·27 (4) | 3470 ± 350 | 1·4 |
| n-Pentanol | 0·35 ± 0·07 (6) | 2700 ± 250 | 1·8 |
| n-Hexanol | 0·36 ± 0·23 (5) | 3540 ± 300 | 1·4 |
| n-Heptanol | 0·42 ± 0·12 (4) | 2520 ± 300 | 1·9 |
| n-Octanol | 0·27 ± 0·03 (4) | 2120 ± 150 | 2·2 |
| n-Decanol | 2·5 ± 1·7 (10) | 2090 ± 150 | 2·1 |
| n-Dodecanol | 4·1 ± 2·7 (8) | 2300 ± 150 | 2·1 |

a. Data include standard deviation and ( ) number of experiments.

b. From $t_L = \dfrac{h^2}{6D}$ (Mean value for h = 0·54 mm)

Fig. 2 shows the effective diffusivities of the permeants in the nail plate tissue as a function of alkyl chain length.

### Theoretical considerations

The nail plate's barrier properties are governed by its anatomical construction and its physicochemical properties and a proposed model must be supportable in terms of both. The model developed here, although speculative, fulfills these requirements. The plate consists of a laminate of sheets of keratinized cells (Caputo & Dadati 1968; Forslind 1970; Forslind & Thyresson 1975). Like the stratum
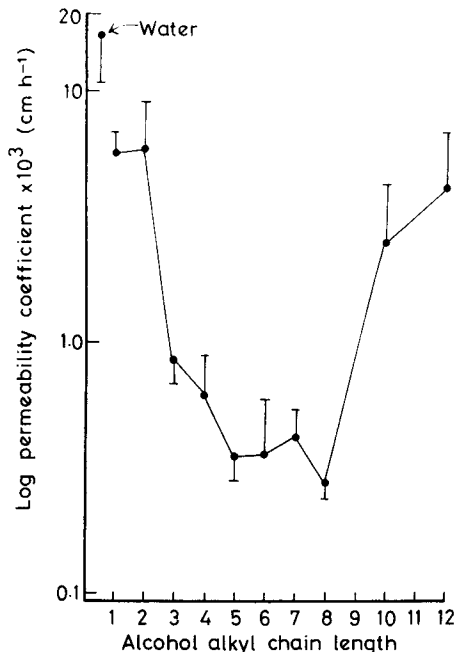
FIG. 1. Relationship between the logarithm of the permeability coefficient and alkyl chain length of the alcohol.

corneum, the cytoplasmic keratin mass is partially crystalline and partially amorphous. In section, thin lipid seams are seen to separate the cell layers. This lipid is from the original cell membranes and is apparently supplemented by intercellular deposition of so-called membrane coating granules during the plate's formation (Hashimoto et al 1966; Hashimoto 1971a,b).
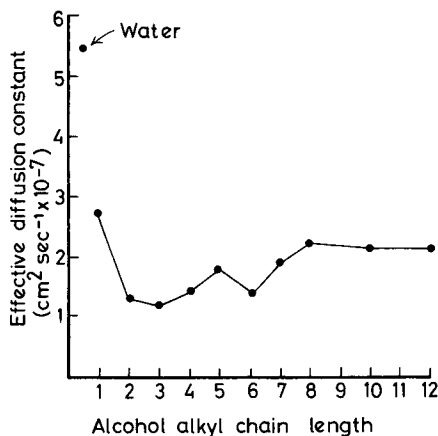


FIG. 2. Effecive diffusivities of the permeants as a function of alkyl chain length of the alcohol.

The above picture allows consideration of diffusion across the nail plate at its first level of organizational complexity. For a given permeant, the principle mass current may either pass directly through the cell units stacked upon one another and separated by the intercellular substance, or may flow mainly around the cell contents by way of the interconnecting, extra-cellular lipid network. The first of these possibilities involves alternating passages through two distinctly different domains. Based on the lipid content of the nail being totally extracellular and 1% of the total volume and on individual cell dimensions of 30 μm diameter (hexagonal) and 1 μm thickness, the latter route would offer a fractional area for diffusion of $5 \times 10^{-4}$. At this percentage composition and with these cell dimensions, the calculated width of the region between cells is approximately 100 Å, in reasonable agreement with ultra-microscopic estimates (Zaias & Alvarez 1968; Hashimoto 1971b). Allowance has to be made for diffusional path of the extracellular route to be tortuous, having a path length up to, but not exceeding, 15 times (1/2 cell diameter), the nail's width.

The following equation can be formulated to describe flux across the nail plate as described using the general principles outlined by Flynn et al (1974):

$$\frac{J}{A} \approx \left[ \frac{f_c}{\Sigma R_c + \Sigma R_M} + \frac{f_L}{R_L} \right] \Delta C \qquad (3)$$

Here J is the total flux (mass/time), A, is the area of application, and $f_c$ and $f_L$ are the fractional areas available for the transcellular and lipid routes respectively. The terms $R_c$ and $R_M$ are the summed resistances of the two types of lamina encountered transcellularly while $R_L$ is the resistance of the extracellular lipid route. The term $\Delta C$ is the driving force for the mass transfer process measured as the concentration difference across the nail plate.

Equation 3 can be made more explicit by including the estimated fractional areas and by defining the resistances in terms of effective thicknesses (h), diffusivities within phases (D), and partition coefficients (K):

$$\frac{J}{A} \approx \left[ \frac{0 \cdot 9995 D_c D_M K_c K_M}{D_M K_M \Sigma h_c + D_c K_c \Sigma h_M} + \frac{5 \times 10^{-4} D_M K_M}{h_L} \right] \Delta C \qquad (4)$$

The subscript c is used to indicate the intracellular protein domain and the subscript M the extracellular

lipid domain. The quantities $h_c$ and $h_M$ are the summed thicknesses of the cytoplasmic laminae and membrane lamellae passed through transcellularly. Added together these yield the total nail plate thickness. Common values of diffusivity $(D_M)$ and partition coefficient $(K_M)$ are given for the lipid elements of the two distinct routes but the $\Sigma h_M \ll \ll h_L$ even in the absence of a significant tortuosity factor. Finally, for all but the most polar permeants, $D_M K_M \Sigma h_c \gg D_c K_c \Sigma h_M$ as $D_c < D_M$ (likely) and $\Sigma h_M \ll \ll \Sigma h_c$. Therefore:

$$\frac{J}{A} \approx \left[ \frac{0 \cdot 9995\, D_c K_c}{\Sigma h_c} + \frac{5 \times 10^{-4} D_M K_M}{h_L} \right] \Delta C \quad (5)$$

Equation 5 gives the flux per unit area for typical permeants in terms of physically meaningful mass transfer parameters. The bracketed quality is a complex mass transfer coefficient or 'permeability coefficient'. Using the symbol P for the permeability coefficient, the statement $J/A = P\Delta C$, applies generally for such mass transfer systems. The operative parallel pathways are indicated by the two separate collections of terms comprising P.

The permeability coefficient profile for a homologous series of permeants will depend upon how the diffusivities and partition coefficients in equation 5 are affected by variation of length of the alkyl chain. It is impossible to predict how $D_c$ and $K_c$ might be affected as very little is known about solubility and molecular mobility in the nail's dense, semicrystalline protein phases. Based on general considerations (Flynn et al 1974) and on partitioning behaviour of long chain fatty acids between the nail plate and water (Baden 1970), $K_M$ may be assumed to follow the general o/w homologue partitioning pattern:

$$\log K_{M.n} = \log K_{M.O} + \pi n \quad (6)$$

where $K_{M.n}$ is the partition coefficient of the homologue of chain length n between a water immiscible phase and water and log $K_{M.O}$ is the Y-intercept of a plot $K_{M.n}$ versus n. The term, $\pi$, is the slope of the log K versus n plot. The nature of the intercellular material is such that $\pi \geqslant 0 \cdot 3$ and therefore $K_{M.n}$ can be expected to grow in an exponential fashion as the alkyl chain is extended.

*Relative permeability of the n-alkanols through nail plate and stratum corneum*
The alkyl chain length dependency of permeability

of the n-alkanols (including water at n = 0) is shown in Fig. 1. From water to n-octanol permeability coefficients decrease systematically and by an overall factor of about 60. This observation rules out the possibility that a lipid pathway is involved for these permeants. Over the same alkyl chain length span diffusivities are also decreasing, but only severalfold. The lag time based diffusivities are on the order $10^{-7}$ cm$^2$ s$^{-1}$, a magnitude which is physically plausible.

A nominal molecular size sensitivity for diffusivity is evident considering the narrow spread in apparent $D_c$ ($D_{effective}$) values. Thus, there must be another cause for the decline in the permeability coefficients with increasing chain length. According to equation 5 partitioning provides the only alternative basis for the declining trend; it would be necessary for the partition coefficients between the protein domain of the nail (keratin) and the external water to decrease about 25-fold. To our knowledge there is no precedent for such behaviour in a mass transfer framework under circumstances where the external media are aqueous. There are, however, some observations supporting the concept that the keratin matrix has a decreasing ability to dissolve the alkanols as the homologous series is ascended. Tillman & Higuchi (1961) note that the solvating and softening abilities of solvents for callus strips are in the order water > methanol >> ethanol. A great deal of work has been done on the sorption of solvents into hair and wool fibres and, according to Harrison & Speakman (1958), the fine structure of wool seems to be inaccessible to molecules larger than n-propanol. On the basis of the present work, it would appear that exclusion associated with increasing hydrophobicity is more a thermodynamic than a kinetic (molecular size) phenomenon. Hair (wool), callus and nail seem to have more in common chemically and physically with each other than they do with the stratum corneum (Baden 1970; Baden et al 1973) and thus inferences drawn from the cited works have good probability of being applicable to the nail. Furthermore, at $10^{-7}$ cm$^2$ s$^{-1}$ the effective diffusivities are too large for an approximately 25-fold factor to be incorporated and hidden in some complex way. It therefore appears that, to a rough first approximation, the nail plate acts as a concentrated hydrogel to the alkanol permeants through n-octanol. The behaviour suggests there is a positive free energy change accompanying the transfer of a methylene group from the external, water medium to the intracellular protein phase.

Skin permeation of the alkanols through n-octanol

is strikingly different (Blank 1964; Scheuplein 1965; Behl et al 1980; Durrheim et al 1980; Flynn et al 1981). As the alkyl chain length is increased, permeability coefficients also increase, with the increase being essentially exponential past ethanol. Such behaviour signifies that the stratum corneum functions for the most part as a hydrophobic membrane to these permeants, with increased permeability coefficients the result of increased partitioning into some critical hydrophobic phase within the horny structure. The stratum corneum's far greater lipid content undoubtedly plays a role here, setting its membrane behaviour apart from that of the nail and perhaps other cornified tissues.

The striking increase in nail plate permeability coefficients from n-octanol to n-dodecanol signals a change in diffusional mechanism. Now permeability is increasing with increased hydrophobicity of the permeants, an unmistakable indication of the emergence of a functionally lipid pathway. Equations 4 and 5 were formulated with such a route in mind, namely a route through the intercellular seams, with the collection of terms, $5 \times 10^{-4} D_M K_{M/hL}$, representing the route's diffusive contribution. Regardless of whether the placement of the lipid pathway is anatomically proper, the route's essential trait is an exponentially increasing distributioning with increased alkyl chain length, as described in equation 6. Even a fractionally minor lipid route will assume rate-controlling proportions at an appropriately long alkyl chain length providing no other competitive pathway has a partitioning sensitivity. The transition to lipid pathway control occurs with but a slight increase in effective diffusivity based on the effective diffusivities of n-decanol and n-dodecanol. It would appear that the route is not tortuous as these effective diffusivities do not allow for great non-linearity in path. The appearance of this route so late on the alkyl chain length profile is consistent with its limited fractional area, which we estimate to be about 1/100 of that of the same pathway in stratum corneum. With a methylene unit partitioning factor $(\pi)$ of $\geqslant 0.3$ as suggested by literature data (Baden 1970), the alkyl fragment necessary to place the permeation process into extracellular control would have to be five to six carbons longer than it would for the stratum corneum, and this is very close to what is experimentally observed. Assuming a fractional area of $5 \times 10^{-4}$ and otherwise using the permeability data in Table 1, the partition coefficient between the extracellular lipid substance and water necessary to account for dodecanol's high permeability coefficient can be estimated, ie, $P \approx f_L D_M K_M/h_M$, and $K_M$ is about 6000. This value is certainly not too large considering the extended length of the alkyl chain.

It is thus evident that the nail plate as a membrane behaves in a manner widely different from the epidermis (stratum corneum). There are other differences. The absolute rate of water transpiration through the nail is faster than through the intact skin ($\approx 10$ times) and, if the rate is thickness-normalized, the ratio is approximately 1000 in favour of the nail. Over a wide polarity range nail plate permeability is inversely related to polarity while the reverse is true for the stratum corneum. The declining nail plate permeability appears related to decreasing affinity of the keratin matrix for the higher alkanols. Attempts to confirm this supposition by way of equilibrium partitioning were not entirely successful.

At extreme hydrophobicities ($\geqslant C_8$) a new pathway for diffusion through the nail becomes evident. And from n-octanol to n-dodecanol equilibrium partition coefficients increased exponentially, a trend generally supportive of the lipid character of the route. A partition coefficient for n-dodecanol of $131 \pm 24$ was obtained. If it is assumed that this $C_{12}$ homologue is concentrated exclusively in the lipid domain, which overall is estimated to occupy approximately 0.01 volume fraction, then dodecanol's intrinsic partition coefficient would be 13 000. This value is only a little over twice the value computed from the permeability coefficient using a fractional area for diffusion of $5 \times 10^{-4}$. Given all uncertainties, this is in reasonable if not fortuitous accord.

It seems likely to us that the alkanol permeability pattern of the nail plate reflects general nail behaviour and thus suggests how other low molecular weight organics might permeate. If this supposition is true, then very polar compounds might be surprisingly easily delivered through the nail plate to underlying tissues. The fact that urea can be used to chemically loosen and separate the nail plate from its bed is a supporting observation (Farber & South 1978). The low incidence of problems associated with the use of powerful hydrophobic organic solvents in nail laquer seems equally reinforcing. Certainly toxic and irritant properties of substances measured via patch tests on skin cannot be extrapolated to the nail. Moreover, physicochemical criteria governing the selection of therapeutic candidates to treat nail disorders would seem to be very different from the established criteria used for drug selection for the skin.

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