THE JOURNAL OF INVESTIGATIVE DERMATOLOGY, 76:76–79, 1981

Vol. 76, No. 2
Printed in U.S.A.

Physiocochemical Characterization of the Human Nail: I. Pressure Sealed Apparatus for Measuring Nail Plate Permeabilities

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Diffusion characteristics of the nail plate are necessary in providing the baselines for rational topical management of nail infections. In order to develop such baselines a unique stainless steel diffusion cell has been designed. The cell permits the exposure of 0.38 cm^2 of nail plate to a bathing medium which is stirred by small motors mounted above the cell. The diffusion of water, methanol and ethanol at constant temperature (37°C), has been examined over periods up to 4 h. Average permeability coefficients of water, methanol and ethanol were determined as $16.5 \pm 5.9 \times 10^{-3}$ cm hr^{-1}, $5.6 \pm 1.2 \times 10^{-3}$ cm hr^{-1} and $5.8 \pm 3.1 \times 10^{-3}$ cm hr^{-1} respectively. Moreover rates of diffusion across the nail were inversely proportional to nail thickness. Based on methanol data, nail plate barrier property appears stable for long periods of aqueous immersion.

Little is known concerning the permeability of the human nail plate yet such knowledge is important to the understanding of toxicities of topically applied chemicals [1,2], to structure/activity considerations in drug design, and to the formulation of topical drug delivery systems and cosmetic systems. A particular problem is that nail is prone to attack by a host of fungi and micro-organisms, some of which invade the living tissues of the nail bed and nail fold and others invade the nail plate itself [3,4]. It is uncertain whether the nail is sufficiently permeable to allow successful topical management of such conditions [6].

In order to assess the feasibility of chemical penetration, a systematic study of the physicochemical factors which govern deposition of topically applied chemicals into nail plate, including definition of molecular size and lipophilicity requirements, seems necessary. There are only a few quantitative measurements of nail plate permeability in the literature [6–9]. This dearth of information may be attributed to the difficulty of working with a relatively rigid membrane of variable thickness. Problems attending construction of a suitable apparatus for the direct measurement of nail plate permeability have yet to be surmounted. The initial purpose of our research was thus to construct a unique diffusion cell to quantitatively assess nail plate permeability.

MATERIALS

Tritiated water* ^{3}H-methanol,† and ^{14}C-ethanol‡ were selected as permeants. Saline (0.9% sodium chloride irrigation solution [2]) was the medium used to prepare the permeant solutions, which were 1×10^{-3} molar or less concentration and thus too dilute to have direct effects on the membrane barrier properties. Cadaver nails were obtained from the University of Michigan, School of Medicine [3].

PERMEABILITY CELL AND METHOD

Stainless steel diffusion cells, which expose 0.38 cm^2 of nail plate to the compartmental solutions, were constructed. The internal and external diameter of the cells were 0.7 cm and 1.3 cm, respectively. The length of each cell half, taken from the edge of the seal, was 3.9 cm. The design allowed a tight seal between the nail and the half-cells using male-female interlocking flanges under pressure (Fig 1). The cell was placed in a clamping device operating as a "c-clamp" (Fig 2) and pressure was exerted about the circumference of a nail plate section cut to fit snugly within the wider flange's diameter. Sample and stirrer ports were provided to each half-cell, the individual volumes of which varied from 1.4–1.6 cm^3. Cells were immersed in individual constant temperature water baths and the contents were stirred with small Teflon propellers attached to Teflon shafts exiting the stirring port. Small Teflon discs with set-pins were used to position the shaft with propeller in the center of the chamber. These also prevented evaporation from this port. The connection of the upper shaft end to the stirring motors (Hurst, 150 rmp§) was with a short section of flexible tubing (Fig 3).

Nails were pre-immersed in normal saline for 24 h before use. Pre-immersion served to clean the nails and also to facilitate trimming to fit the diffusion cells. Nail thicknesses were measured with a micrometer prior to insertion in the diffusion cells.

After assembly of a cell, the 2 compartments were filled with either normal saline or citrate buffer and the stirring shafts were connected to the stirring motors. Citrate buffer was only used during some determinations of ethanol permeability. No differences in permeability rate were observed when the buffer was employed. The contents were mixed for 30 min and background samples were taken. The donor compartment was then charged with a concentrate of the radiochemical permeants and the run commenced. Five minutes were allowed for uniform mixing and then the "zero time" donor sample was withdrawn. Samples were taken from the receiver compartments at predetermined intervals up to 4 h. At the end of a run a second donor sample was taken. The mean of the initial and final donor samples was used as the effective concentration differential across the nail over the course of the experiment. The late donor sample was never less than 15% of the "zero time" donor sample. Samples were taken from the water bath before and after the run to check for cell leakage.¶

The samples were added to scintillation cocktail and counted on a Beckman LS 9000 liquid scintillation counter. The experiments were performed at 37°C. In all cases a dual label procedure was followed. The scintillation counter employed is equipped with energy windows which, when properly set, allow the separation of low energy emissions (predominantly ^{3}H) from the higher energy emissions of carbon-14 [10]. When concentrations of the radioisotopes are in certain proportions, it is possible to accurately factor the total disintegrations into its component tritium and carbon-14 contributions.

The permeability of ^{3}H-methanol was also studied as a function of duration of immersion of the nail in saline. Experiments were performed sequentially over a 49-day period. Between runs the nail was stored in saline solution.

Manuscript received May 13, 1980; accepted for publication July 26, 1980.

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* New England Nuclear, Boston, Massachusetts.

† Abbott Laboratories, North Chicago, Illinois.

‡ Generously supplied by Dr. T.M. Oelrich, Department of Anatomy.

§ Hurst, Mfg. Co. Ltd., Princeton, Ind.

¶ The seal on the nail edges should be sufficient to preclude intercompartmental leakage. However, leakage around the membrane edges can also be discounted on the basis that large differences (20-fold) occur in the permeability rates of homologues with only slight variation in molecular volumes. For example, the permeability coefficients for propanol and octanol are 0.83 ± 0.15 and $0.27 \pm 0.03 \times 10^{-3}$ cm hr^{-1} respectively. These data will be fully reported in a later article.

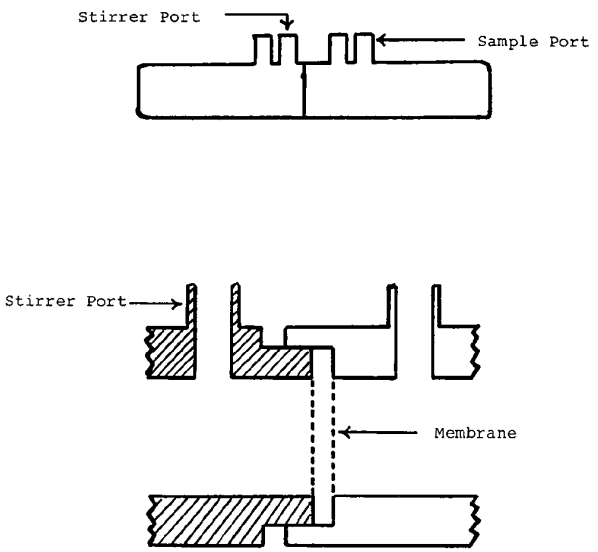

FIG 1. Cross-section of the interlocking flange of the diffusion cell

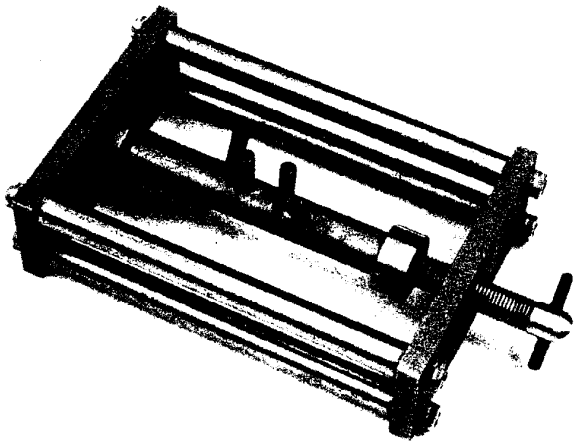

FIG 2. The diffusion cell and clamping device.

DATA ANALYSIS

After factoring the data into its tritium and carbon-14 components, the individual data were plotted as counts (amount of permeant) collected in the receptor compartment as a function of time (Fig 4). Correction was made for sampling, which in all cases was done with replacement using normal saline. The permeability coefficient for a given run was calculated from:

$$J = P.A.\Delta C = V\frac{dC}{dt} \qquad \text{Eq. 1}$$

where:

- J = the total flux or amount penetrated vs time, cpm hr^{-1} or cpm/hr, in the pseudo-steady-state regions of plots such as in Fig 4
- P = the permeability coefficient (cm/hr)
- A = the cross-sectional area of the membrane (cm^2)
- ΔC = the concentration differential across the membrane as described above
- V = volume of the half-cell into which the permeants were collected
- $\frac{dC}{dt}$ = actual pseudo-steady-state slope of plots as in Fig 4 in cpm/hr/cm^3.

RESULTS

The permeability coefficients for ^{3}H-water, ^{3}H-methanol and ^{14}C-ethanol are given in the Table. These are provided with standard deviations and the number of experiments with each compound. Also tabulated are thickness normalized permeability coefficients. Figure 5 shows the effect of nail hydration time on the permeability of methanol. The data are for a single nail placed in a diffusion cell at the indicated times and otherwise kept in fresh saline. Figure 6 indicates how the permeabilities of methanol and ethanol were influenced by nail thickness.

DISCUSSION

Several investigators have studied the water permeability of nail plate [6–9]. Burch and Windsor [8] report water fluxes of 1.16–5.30 mg/cm^2/hr, through toenails, depending upon temperature and humidity. They assessed weight loss of a brass cylinder, filled with saline and closed at one end by nail plate. Weight loss was assumed to be due to loss of water through the nail. Using a similar method Baden, Goldsmith, and Fleming [6] reported that the flux of water across the nail ranges between 2.0–3.0 mg/cm^2/hr. Spruit [7] designed a moisture sensing device which noninvasively measures insensible perspiration. He found the nail water vapour loss to average 2.40

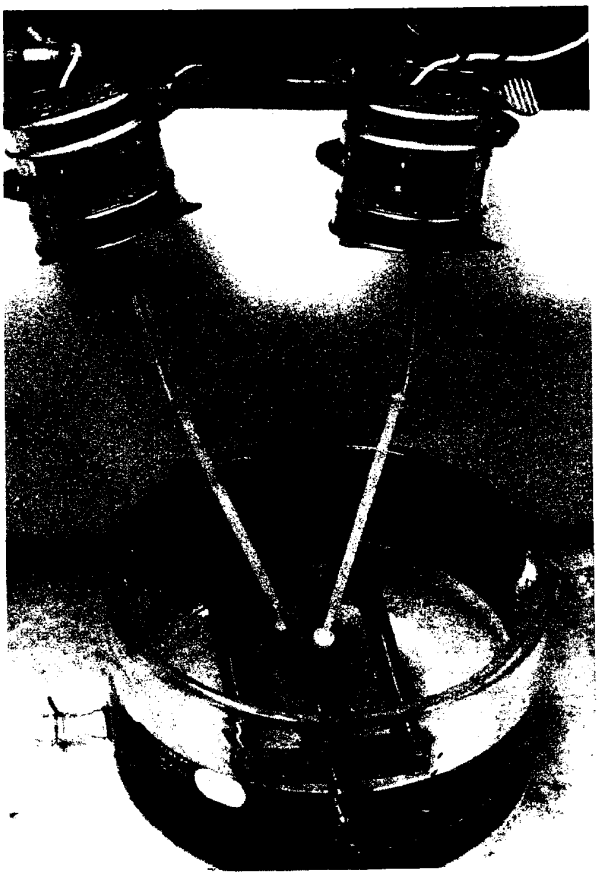

FIG 3. The diffusion cell, water bath and stirring motors.

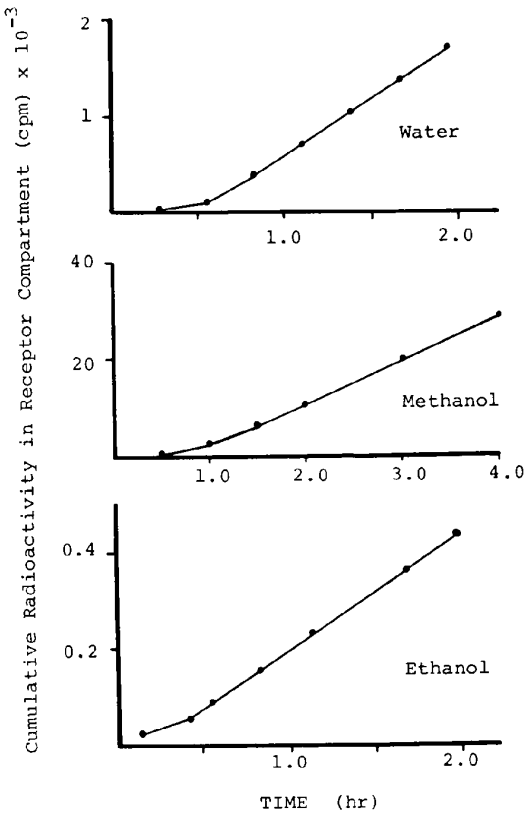

FIG 4. Typical nail permeation plots for water, methanol and ethanol.

TABLE *Permeability coefficients*

Permeant	Permeability[a] coefficient (× 10^3) cm hr^{-1} ± SD	No. of observations	Normalized[b] permeability coefficient	No. of observations
^{3}H-water	16.5 ± 5.9	22	10.5 ± 3.5	12
^{3}H-methanol	5.6 ± 1.2	26	5.1 ± 1.1	18
^{14}C-ethanol	5.8 ± 3.1	26	2.7 ± 0.8	26

[a] *P* observed.
[b] *P* observed × nail thickness (mm).

mg/cm^2/hr, in close agreement with the earlier *in vitro* results. Moreover, Spruit found that when the thickness of the nail was accounted for, there was little variability in the permeability rate. The observed rate is about 10 times greater than insensible perspiration measured across normal skin. This actually suggests that nail plate is up to 1000 times more intrinsically permeable to water than stratum corneum because the ratio of thicknesses of these tissues is roughly 100 (nail over stratum corneum).

In the present experiments a water flux of 12.6 ± 5.8 mg/cm^2/hr was obtained (calculated from total water transported over the experimental run). This is about 5-fold greater than literature values. However, the previous investigators worked with nails with a dry, dorsal surface. The present results appear to be the first assessements of rates across hydrated, totally immersed nails. Consideration must be given to the possibility that hydration of the dorsal surface increased the rate of water's diffusion across this layer of the nail. Regardless, agreement with previous work is generally satisfactory.

The observation that the permeability rate of methanol across the nail does not evidence systematic change over a period of up to 49 days (Fig 5) suggests that, after the initial 24 h soaking, no further significant hydration occurs in this membrane. The upper limit of the nail plate's capacity for water appears to ba about 25% [6] and is only 2–3 times greater than the normal water content of the nail, which is estimated to lie between 7 to 12% [6,11]. The approximately 5-fold greater flux of water reported here relative to that observed by others using a nail plate with a dry dorsal surface seems a reasonable consequence of full hydration. The relative constancy of methanols permeability coefficient over a 49-day period suggests that the hydrated nail is a stable barrier and is able to withstand the repeated application of pressure without severe detriment.

The rate of diffusion of methanol and ethanol is clearly dependent on nail thickness (Fig 6). This is a general expectation as, for a simple isotropic membrane, the permeability rate is related to membrane thickness by

$$P = \frac{KD}{h} \qquad \text{Eq. 2}$$

providing boundary layers are insignificant. In this expression K is the membrane—solution partition coefficient of the per-

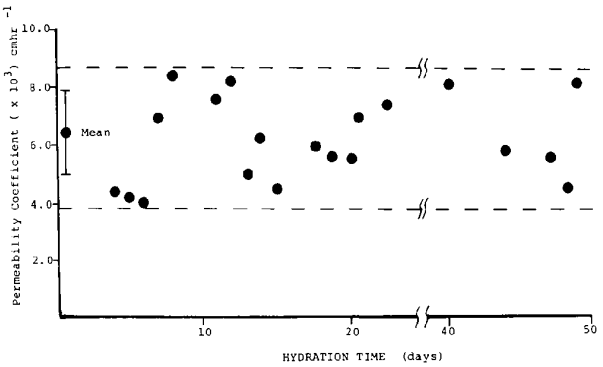

FIG 5. The effect of nail hydration time on the permeation of methanol.

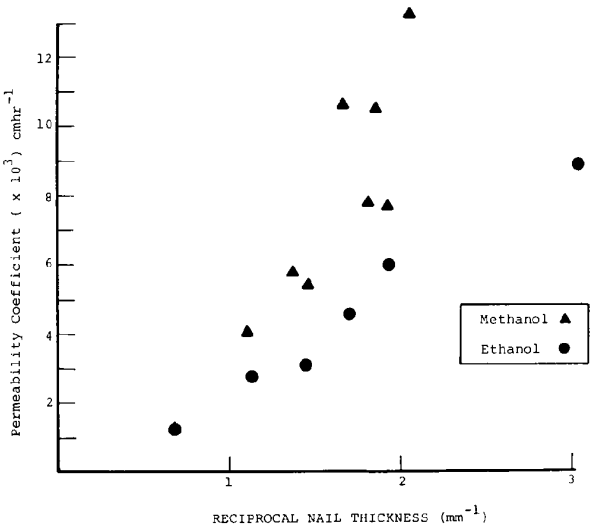

FIG 6. The relationship between nail thickness and permeation rates of methanol and ethanol.

meant, D is the diffusion constant of the permeant within the membrane, and h is the membrane thickness. The fact that the reciprocal dependency on thickness is not perfectly obeyed for either permeant suggests that either the substrata of the nail have somewhat different permeabilities and proportions or conditions which cause nails to thicken also lead to a less permeable plate on a per unit thickness basis. In this study a total of 14 nails were used ranging in thickness from 0.33 mm to 1.43 mm, and all were either finger or thumb nails, too few to make a firm conclusion as to the exact shape of the profile and the causes of the nonlinearity. The data presented here are nevertheless indicative of a substantial thickness dependency (for methanol $p < 0.01$; for ethanol $p < 0.001$).

In summary, a diffusion cell has been designed which is useful in the quantitative measurement of human nail plate permeabilities. Preliminary studies using the diffusion cell indicate that the unique pressure-seal used in this apparatus is without detrimental effect on the barrier properties of the plate.

We should like to thank Johnson and Johnson (Ortho) Inc. for financial support.

REFERENCES

1. Sulzberger MD, Rein CR, Fanburg SJ, Wolf M, Shair HM, Popkin GL: Allergic eczematous reactions of the nail bed: Persistent subungual & ungual changes based on contact with 'undercoats' containing artificial resins and rubber. J Invest Dermatol 12: 67–72, 1948
2. Shelley WB: Onycholysis due to topical 5-fluorouracil. Acta Dermatovener (Stockh) 52: 320–322, 1972
3. English MP: Nails and Fungi. Br J Dermatol 94: 697–701, 1976
4. Sagher F: Histological examinations of fungus infections of the nails. J Invest Dermatol 13: 338–357, 1949
5. Baranov AF, Marochkina IA, Konopikhina GA, Kolokolova NV, Jakimenko DV, Panova PM: Experiences in treatment of onychomycoses with keratolytics and fungicidal plaster. Vestn Dermatol Vener 36: 65–67, 1962
6. Baden HP, Goldsmith LA, Fleming B: A comparative study of the physicochemical properties of human keratinized tissues. Biochim Biophys Acta 322: 269–278, 1973
7. Spruit D: Measurement of water vapor loss through human nail *in vivo*. J Invest Dermatol 56: 359–361, 1971
8. Burch GE, Windsor T: Diffusion of water through dead plantar, palmer and torsal human skin and through toenails. Arch Dermatol Syph 51: 39–41, 1946
9. Jacobi O: Die nägel des lebenden menschen und die perspiratio insensibills. Arch Klin Expt Dermatol 214: 559–572, 1962
10. LS 9000 Instruction Manual, Beckman Instruments, 1979.
11. Forslind B: Biophysical studies of the normal nail. Acta Dermatovener (Stockh) 50: 161–168, 1970