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Structural characteristics and permeability properties of the human nail: A review

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Synopsis

The human nail forms a resistant barrier to the topical penetration of actives. Thus, treatment of nail disorders, such as fungal infections, remains a challenge because of the difficulty encountered in achieving therapeutic concentrations of drugs at the site of infection. The nail is primarily composed of a highly cross-linked keratin network that contains several disulfide linkages. This unique structure results in a highly effective permeability barrier. Nail penetration has been reported to be affected by molecular size and hydrophilicity; smaller, water-soluble molecules are found to preferentially permeate the nail. Permeation of undissociated drugs is favored in certain instances. Also, some studies indicate that the nature of the vehicle can influence drug penetration. Recent research has focused on improvement of penetration of topically applied actives into and through the nail. Studies have shown that compounds containing sulfhydryl groups in conjunction with keratolytic agents can significantly enhance drug penetration, relative to a control formulation (without enhancer). Such sulfhydryl compounds are thought to reduce the disulfide linkages in the nail keratin matrix. Thus, although some success has been achieved in enhancing penetration of drugs through the nail, further research is required to achieve successful topical products for treatment of nail infections.

INTRODUCTION

The human nail acts as a protective covering to the delicate terminal phalanges of the fingers and toes and helps in grasping small objects. Changes in the appearance of the nail result from a variety of conditions such as fungal, bacterial, and viral infections or dermatological disorders (1–3). Various cosmetic procedures such as application of artificial acrylic nails, use of nail hardeners, and manicures can also result in nail disorders (4).

Fungal infections of the nail, called onychomycosis (OM), are some of the most commonly encountered dermatological disorders, typically manifested as localized infections of the nail or nail bed. OM has widespread incidence and is thought to account for 40% of all nail disorders. It is estimated that 4.9–12.3 million people are affected with OM in the United States. The incidence of OM increases with age, and toenails are infected about seven times more frequently than fingernails. Mycotic nail infections are caused by dermatophytes, yeasts, and nondermatophyte molds, although dermatophytes are believed to be the principal causative organisms in OM (3,5). Symptoms of OM include

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discoloration of the nail, brittleness, pitting, splitting, hypertrophy, or even complete separation of the nail from its bed (onycholysis) (6). Thus, nail diseases may result in unaesthetic changes in nail appearance. Hence, these disorders require treatments that will eradicate the infection and allow the nails to return to a cosmetically acceptable state.

Current treatment modalities for OM include surgery and oral antifungals. While surgical nail removal (avulsion) is invasive and painful, high doses of oral medication can lead to systemic side effects. These adverse effects combined with treatment times extending to several months, and frequent incidences of relapses observed with oral antifungals, often lead to patient noncompliance and interruption of therapy. Thus, topical therapy is the most desirable, but it has met with limited success to date. The primary reason for the resistance of the nail to topical therapy is the extremely low permeability of the nail plate and thus the inability of actives to reach the site of infection (7). This review will outline the structure, chemical composition, and physical properties of the nail, and will also describe studies used to investigate and improve permeation of actives through the human nail.

STRUCTURAL CHARACTERISTICS OF THE HUMAN NAIL

STRUCTURE AND ANATOMY OF THE NAIL

The nail, shown schematically in Figure 1, consists essentially of the hard, flat, and roughly rectangular nail plate, which is closely connected to the nail bed. The nail bed appears pink in color due to its extensive vascular network, and can be seen due to the translucency of the nail plate. The nail wall surrounds the nail proximally, while the groove-shaped nail fold encloses the nail laterally. The nail root lies 3–5 mm deep within the nail fold and is invisible. The nail plate emerges from the matrix, the distal end of which appears as the whitish crescent-shaped lunula. The eponychium is formed from the epidermis of the proximal nail wall. The stratum corneum of the eponychium forms the cuticle. Below the cuticle lies the matrix. The skin under the free edge of the nail is called the hyponychium (8–9).

The matrix. The matrix is mainly responsible for the formation of the nail plate. During nail plate formation, the basal cells of the matrix flatten, and fragmentation of cell nuclei and condensation of cytoplasm occur to form flat, keratinous cells whose cell borders, in contrast to hair, are retained. The lower cell layers of the matrix contain melanocytes, and this may cause varying degrees of pigmentation of the human nail plate, depending on race (8–9).

The nail bed. The nail bed extends from the lunula to the hyponychium. It does not contribute much to the formation of the nail plate; however, keratin production in the nail bed occurs synchronously with extension of the nail plate. The nail bed acts mainly as a holder and slide for the nail plate (8–9).

The nail plate. The nail plate consists of dead, cornified, adherent cells without nuclei, but with prominent cell borders. The nail plate is 0.5–1.0 mm thick, made of α-keratin, and consists of three layers: the dorsal and intermediate nail, formed from the matrix; and the ventral nail, formed from the nail bed (Figure 1). The dorsal nail is a few cell layers thick and contains hard keratin, while the intermediate nail contains softer keratin

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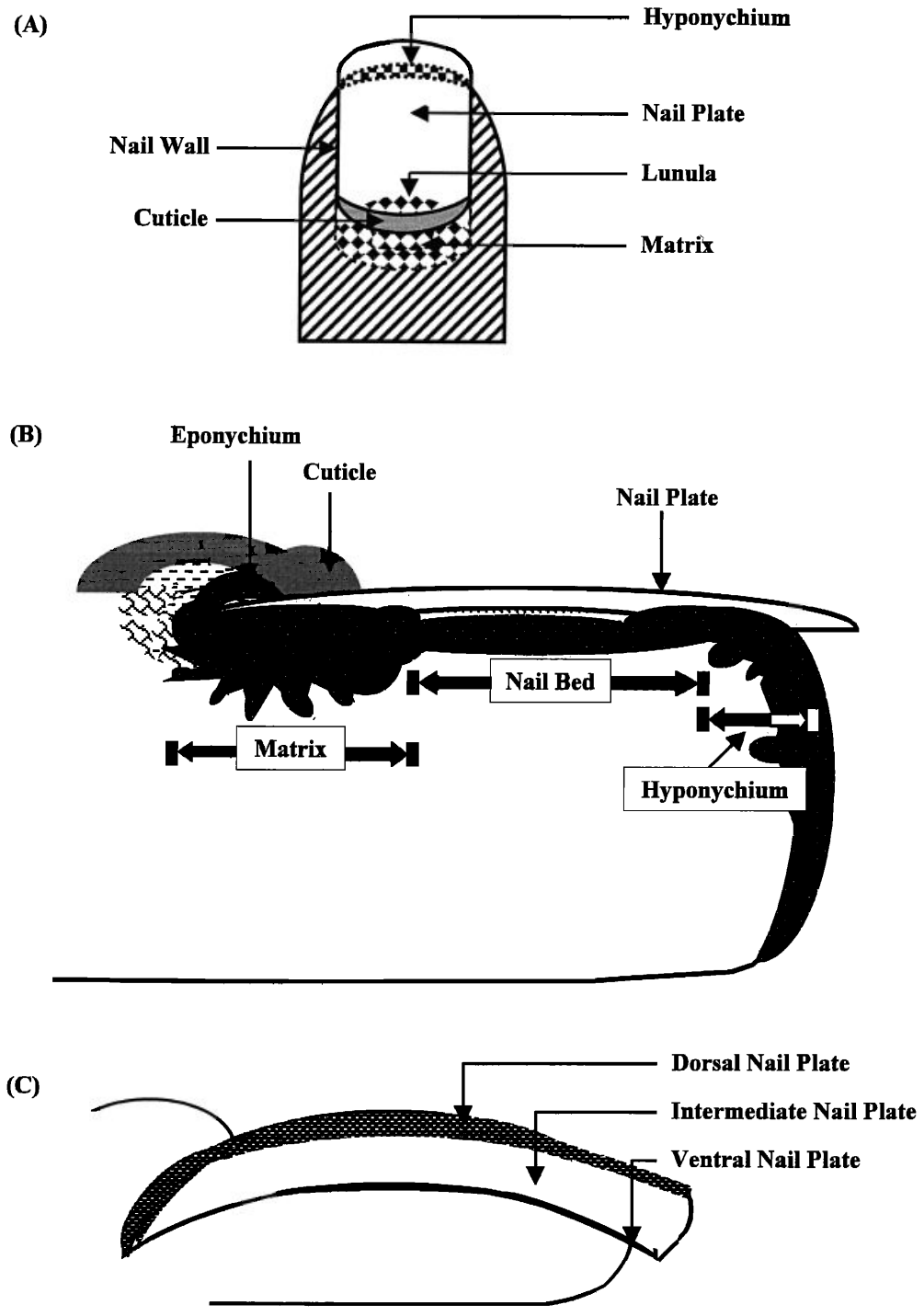


Figure 1. Schematic diagram of the human nail. A: top view. B: longitudinal view. C: longitudinal section of the nail plate. Adapted from references 5, 9.

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and accounts for three fourths of the whole nail thickness. The ventral nail is one to two cell layers thick and is comprised of soft, hyponychial keratin. The ventral nail serves to connect the nail plate securely to the substratum (9).

BLOOD SUPPLY TO THE NAIL

The nail matrix and nail bed have a rich blood supply that originates from two main arterial arches lying below the nail plate. In addition, there is an extensive capillary blood supply to the tissues around the nail; in particular, a capillary loop system supplies blood to the whole nail fold. Furthermore, the nail bed has a rich supply of lymphatic vessels and glomus bodies that are thought to be involved in regulating blood supply to the extremities in cold weather. Despite this extensive vascular network, defective peripheral circulation may be one of the main causes of nail deformities (10).

GROWTH OF THE NAIL

The nail is formed continually from the matrix, unlike hair, which is formed cyclically. The growth rate depends on matrix cell turnover. Toenails grow 30–50% more slowly than fingernails. Typically, the fingernail grows at an average rate of 3 mm per month, and nails of the dominant hand (i.e., right vs. left) exhibit a faster rate of growth. Growth rate of the nail is usually independent of nail thickness. However, growth occurs in a definite order in relation to the length of the fingers; in general, the longer the finger, the faster the growth. Various pathological conditions can cause either an increase or decrease in growth rate. The regeneration time of the nail is dependent on growth rate. The fingernails usually have a total regeneration time of about 160 days, while toenails take approximately a year to regenerate. Due to long regeneration times, treatment of nail diseases requires several months (9).

CHEMICAL COMPOSITION OF THE NAIL

The main chemical constituent of the human nail is keratin, a scleroprotein containing large amounts of sulfur. Both epidermal- and hair-type keratins are present, although the latter make up more than 90% of the protein content. Recent studies using double-label immunofluorescence techniques have shown that the nail matrix contains three populations of cells: cells expressing either skin or hair type keratins and cells expressing both keratins (11–12). The α-keratin filaments (helical) interact with the cystine-rich non-helical matrix keratins, and are oriented perpendicular to the direction of nail growth and parallel to the free edge of the nail. This alignment of keratin filaments and the strong adherence of nail cells to each other are thought to contribute to nail hardness (8–9).

The nail contains various amounts of amino acids that condense together to form the polypeptide chains present in keratin. The amino acid composition of human nails is shown in Table I. For comparison, the amino acids present in human hair and stratum corneum, as well as those in sheep horn, hoof, and wool are included. As seen from Table I, the content of glycine and half-cystine in human nails is similar to that of human hair and sheep wool, rather than human stratum corneum. However, the amino acid com-

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Table I
Amino Acid Composition (as residues per 100 residues) of Human Nail, Human Hair, and Human Stratum Corneum, Compared With That of Sheep Wool, Horn, and Hoof

	Human			Sheep		
Amino acid	Nail	Hair	Stratum corneum	Wool	Horn	Hoof
Lysine	3.1	2.5	4.2	2.7	3.8	4.0
Histidine	1.0	0.9	1.5	0.8	1.3	0.9
Arginine	6.4	6.5	3.8	6.2	6.7	7.2
Aspartic acid	7.0	5.4	7.9	5.9	7.8	8.4
Threonine	6.1	7.6	3.0	6.5	4.8	5.0
Serine	11.3	12.2	13.6	10.8	9.6	9.5
Glutamic acid	13.6	12.2	12.6	11.1	12.9	13.7
Proline	5.9	8.4	3.0	6.6	3.8	4.0
Glycine	7.9	5.8	24.5	8.6	11.1	9.1
Alanine	5.5	4.3	4.4	5.2	5.9	6.4
Valine	4.2	5.5	3.0	5.7	5.2	5.7
Methionine	0.7	0.5	1.1	0.5	0.8	0.8
Isoleucine	2.7	2.3	2.7	3.0	3.3	3.6
Leucine	8.3	6.1	6.9	7.2	9.1	9.5
Tyrosine	3.2	2.2	3.4	3.8	5.0	4.0
Phenylalanine	2.5	1.7	3.2	2.5	2.7	2.7
Half-cystine	10.6	15.9	1.2	13.1	6.2	5.7
Sulfur (%)	3.2	4.5	1.4	3.8	2.1	2.2

Adapted from references 13, 14.

position of human nails differs considerably from that of horn or hoof derived from sheep. In general, significant amounts of glutamic acid, half-cystine, arginine, aspartic acid, serine, and leucine are present in human nails (9,13–15). The nail cellular envelope contains large amounts of proline, unlike the cell matrix, which contains numerous cysteinyl residues (16). Carbohydrates are also found to be present in the cell membrane complexes of human nails. Allen *et al.* (17) demonstrated by formic acid extraction and lectin-binding studies that sugar characteristics of membrane glycoproteins (i.e., mannose, galactose, N-acetylglucosamine, and N-acetylgalactosamine) were present in the cell membrane complexes of nails.

The lipid content of nails is less than 5% (8–9), while the sulfur content is high, as in hair (Table I) (13–4). Nitrogen is another major component, resulting from the presence of high levels of keratin. The nail also contains low levels of Ca, Mg, Na, K, Fe, Cu, and Zn (18). The calcium content was earlier thought to be responsible for the hardness of the dorsal nail (19). However, it is now believed that the presence of disulfide linkages, rather than the calcium content, is responsible for nail hardness (8–9). Trace amounts of Cr, Se, Au, Hg, Ag, and Co have also been reported in human thumbnails (20).

STRUCTURE OF KERATIN

The main form of keratin present in all mammalian hairs, wool, horns, claws, nails, and quills is α-keratin. The presence of covalent disulfide cross-links, which occur due to the high proportions of cystine, confers an exceptional degree of physical and chemical stability on the keratins. In addition to the intermittent disulfide cross-links, there are

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other secondary bonds also present in the keratin structure. These include Van der Waals interactions, hydrogen bonds, and coulombic interactions. The coulombic interactions arise because of the presence of negatively charged carboxylic acid groups (COO^-) and positively charged amino groups (NH_3^+) formed on the side chains of acidic and basic residues. These secondary bonds and interactions play an important role in maintaining molecular order in the keratins, which, in turn, control the freedom of movement and cooperation between molecular chains forming the keratin structure. These characteristics of keratins greatly influence the physical and mechanical properties of the nail (21).

X-ray diffraction studies have revealed a high degree of order (crystallinity) in the keratin structure. The keratinous tissue is primarily composed of α-keratin filaments (7–10 nm in diameter and several micrometers in length) embedded in an amorphous matrix. The α-helix form constitutes about 35–55% of the keratin, and the rest is the non-helical amorphous form. The α-helix portions have a low sulfur content; however, the amorphous sections are high in disulfide linkages (22–23). Apparent molecular weights of low-sulfur and high-sulfur proteins (estimated by electrophoresis) were reported to be in ranges of 55,000–76,000 and 26,500–43,000 daltons, respectively. The isoelectric points for hair and nail low-sulfur protein components are in the range of 4.9–5.4 (24). The term "α" refers to the typical high-angle X-ray diffraction pattern obtained for α-keratin filaments. Two reflections are obtained that are diagnostic for α-keratins. The 0.516-nm reflection on the meridian corresponds to a repeat in the fiber direction, and the 0.98-nm reflection on the equator corresponds to a spacing repeat at right angles to the fiber direction (21,25).

PHYSICAL PROPERTIES OF THE NAIL

The water content of the nail is normally about 18% (9). Early work showed that the rate of diffusion of water through toenails was essentially the same as that through the palms and soles. Moreover, increases in temperature or air currents led to increased diffusion, whereas changes in the humidity of the air influenced water diffusion to a smaller extent (26). Further studies on the water content of nails have revealed that nails are similar to hair in that they are much more permeable to water than the stratum corneum. Also, the uptake of water and rate of water loss seem to be unaffected by prior lipid extraction of the nails with a 3:1 chloroform:methanol mixture. This indicates that the lipid component of nails does not limit water loss from them. The modulus of elasticity of nails has been measured and is found to be dependent on water content of the specimen (27). Forslind and coworkers (28) measured effective elastic modulus (independent of particular dimensions of a nail specimen) and concluded that the water content of fingernails affects their rigidity and stiffness, while natural nail curvature does not affect effective elastic modulus. These authors indicate that contact with detergents, organic solvents, oils, etc., may also influence nail stiffness. In another study, the specific water vapor loss (a product of water vapor loss and nail thickness) was found to be independent of fingernail thickness (29).

Nail flexibility has also been assessed, and has been found to be directly related to water content. Finlay *et al.* (30) used a specially developed nail flexometer to assess changes in properties of the nail *in vitro* after treatment with various agents. The flexometer specifically measured the ability of the nail specimen to withstand repeated flexions, and

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was thus a measure of nail flexibility. They found that during water immersion of nails, nail weight increased by 22% of its original value in two hours and then unexpectedly decreased. Flexibility of nails continuously increased with water immersion. Treatment of nails with mineral oil showed no increase in flexibility; however, mineral oil applied to previously hydrated nails prolonged their flexibility. A phospholipid-water preparation was also found to increase nail flexibility of normal and lipid-extracted nails. It was postulated that this increased flexibility may be due to the ability of the phospholipids to bind water.

In a more recent study, transonychial water loss (TOWL) was measured using an evaporimeter in 21 healthy adults, and was reported to be in the range of 1.17–3.35 $mg/cm^2/h$. This rate of water loss through the nail plate was not significantly different from the transepidermal water loss (TEWL) from the dorsum of the hand. The TOWL appeared to decrease with age and was not affected by nail plate thickness (31).

PERMEABILITY OF THE HUMAN NAIL

By virtue of its chemical composition, the nail plate forms an effective barrier to the permeation of drugs. Thus, the diffusion of actives into and through the nail is extremely poor relative to other membranes such as the skin. Hence, topical medication for treatment of nail infections has been ineffective to date, and information on nail permeability still remains limited. Currently, only oral antifungal therapy is approved for treatment of OM in the United States. However, since efficacious topical nail therapy is most desirable, recent research has focused on characterizing and improving the permeation of drugs through the human nail. Some of the permeability properties and advances made in topical therapy of nail diseases are discussed in the remainder of this review.

EFFECT OF MOLECULAR WEIGHT AND LIPOPHILICITY OF THE PERMEANT

Walters *et al.* designed the first diffusion cell, specially adapted to quantitatively measure nail plate permeability *in vitro,* in 1981 (32). This stainless steel diffusion cell permitted the exposure of 0.38 cm^2 of the nail plate to the donor and receiver solutions, present on either side of the nail. The flux and permeability coefficients of the permeants, based on Fick's Law, were reported. A water flux (determined by monitoring the permeation of tritiated water through cadaver nails) of 12.6 ± 5.8 $mg/cm^2/h$ was obtained using this diffusion cell. This flux value was about five times higher than that reported by earlier researchers. This was explained by the fact that nails used in this study were hydrated, in contrast to dry nails used in earlier investigations. To check the integrity of the nail permeability barrier as a consequence of hydration, the permeability coefficient of methanol was monitored over a period of 49 days and was found to remain fairly constant. This indicated that the nail was a stable barrier and was not affected by the repeated application of pressure required to seal the nail in the diffusion cell. Furthermore, the nail plate permeability was found to be almost inversely proportional to nail thickness.

These diffusion cells were later used to study the *in vitro* permeation of a series of dilute, homologous alcohols (from methanol to *n*-dodecanol) through the nail plate. A radioactive assay was used to analyze concentration of the active, and permeability coeffi-

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cients, lag times, and effective diffusion coefficients were calculated. The effect of increasing chain length on the permeability coefficients and diffusion coefficients of the *n*-alkanols is depicted in Figure 2. Figure 2 shows that the permeability coefficients and diffusion coefficients of the alcohols from methanol through *n*-octanol decreased with increasing chain length. The lag times also showed a corresponding increase (not shown). The authors thus concluded that the nail plate appeared to behave as a concentrated hydrogel in this case, and that the partitioning of the alcohols into the complex keratin matrix decreased with increasing hydrophobicity up to *n*-octanol. However, with the higher alkanols from *n*-octanol to *n*-dodecanol, it was found that the permeability coefficients through the nail plate increased exponentially (Figure 2). The authors suggested the involvement of a possible parallel lipid pathway to explain the permeability result for extremely hydrophobic molecules (33). Neat (undiluted) alcohols were also found to demonstrate trends similar in permeation to the dilute alcohols. However, the permeability coefficients of the undiluted alcohols (except methanol) were about one fifth those of their dilute counterparts. It was observed that the rate of transfer for the lower alcohols increased through the lipid-extracted (with a 3:1 chloroform:methanol mixture) nail plate. On the other hand, a sixfold decrease in the permeation rate of *n*-decanol was observed through the delipidized nail. The authors suggested that the decrease in permeation rate observed for the larger alkanols resulted because the rate-

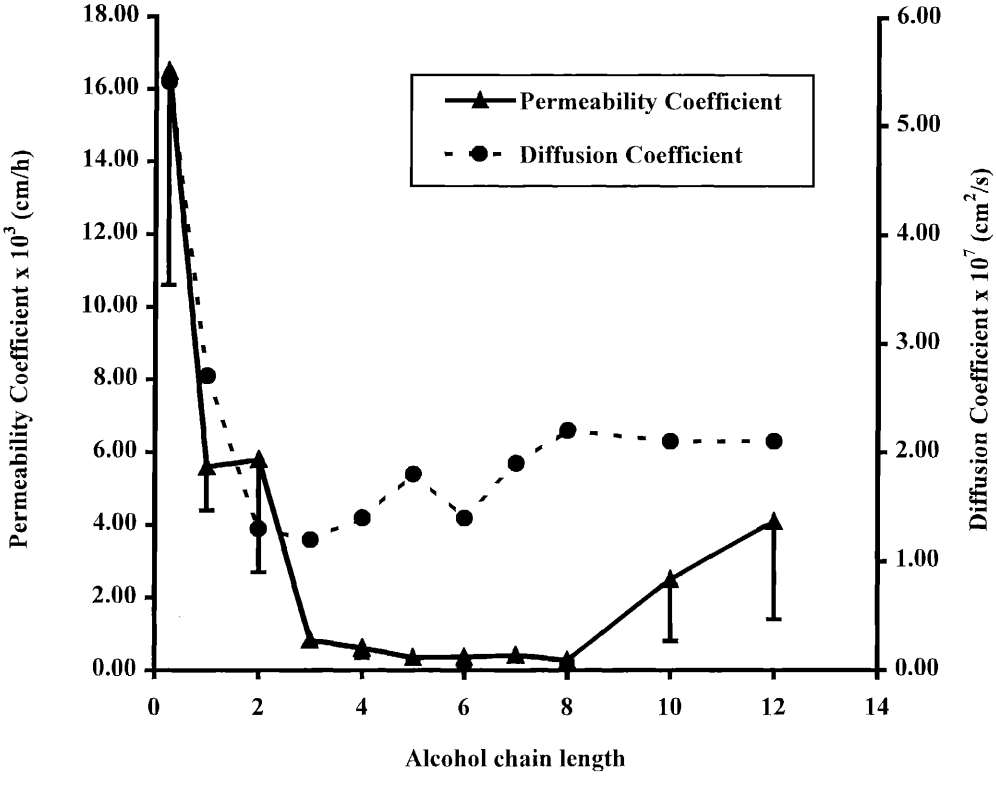


Figure 2. Effect of chain length on the permeability coefficients and diffusion coefficients of a series of diluted, homologous *n*-alkanols through the human nail plate. Mean ± S.D., n = 4–26. Adapted from reference 33.

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controlling lipid pathway for the higher alcohols was interfered with by extraction of the very low, but necessary, lipid level in the nail plate (34).

In a study by Soong (35), the *in vitro* penetration of acetic acid (molecular weight = 60), benzoic acid (molecular weight = 122), and suprofen (molecular weight = 260) through nails was investigated. Steady-state conditions were achieved in these experiments, and the permeated drug was assayed by HPLC. The results of this study showed that as the size of the permeant increased, the lag time increased and diffusion coefficients decreased. The data from this detailed investigation indicated that small, lipophilic molecules would be good candidates for topical nail therapy, both in terms of shorter lag times and faster penetration into the nail plate (due to effective drug partitioning). However, the author cautions against using a very highly lipophilic molecule since water has been shown to facilitate drug transport across the nail plate (33). In order to ascertain whether the dorsal part of the nail plate (which contains tight intercellular junctions) constituted the main barrier to drug penetration, the permeability of benzoic acid through intact as well as shaved (dorsal part removed by scraping) nails was studied (35). No dramatic increase in the permeability coefficient occurred with the shaved nails (which was expected if the dorsal nail constituted the main barrier). Thus, the author speculated that the intracellular structured keratin was the main barrier to the permeation of drugs.

A recent study has further attempted to explain the transport mechanism of permeants through the nail. In this investigation, Mertin and Lippold (36) studied the *in vitro* permeation of homologous nicotinic acid esters (with octanol/water partition coefficients ranging from 7 to >51000) through human nail and a keratin membrane derived from bovine hooves. The diffusion cells used by these researchers were of the Franz-type, temperature was maintained at 32°C, and drug analysis was performed spectrophotometrically for studies with the hoof membrane, and by HPLC for studies with the nail plate. It was found that the permeability coefficients through both human nail and bovine hooves did not increase with increasing partition coefficient or lipophilicity. Thus, the authors stated that the nail appears to behave as a hydrophilic gel membrane rather than as a lipophilic partition membrane. Penetration of acetaminophen and phenacetin showed that maximum drug flux was a function of solubility in water or the swollen keratin matrix. This study showed that water solubility of the drug is an important consideration in formulating a topical product for nail disorders, as increased water solubility can enhance maximum drug flux.

The relationship between the molecular weight (MW) of several drugs (including a series of antimycotics) and permeability through the nail plate and hoof membrane was also investigated (37). The donor compartment consisted of a drug suspension in ethanol (42% v/v), pH 8.1. Due to the limited aqueous solubility of antimycotics, the same medium (without drug) was used as the receptor fluid. The effect of molecular size on the permeability coefficient of antimycotics through the hoof membrane (P_{Hoof}) is depicted in Figure 3. A linear relationship with a negative slope between log P_{Hoof} and log MW was reported for the antyimycotics (Figure 3). Similar trends were seen with other drugs (nicotinic acid esters, acetaminophen, phenacetin, etc.) for permeability coefficients through both nail plate (P) and hoof membrane (P_{Hoof}). However, the permeability coefficients of the drugs tested were higher through the hoof membrane than through the nail in all cases. This was considered to be due to the denser keratin network (and therefore fewer pores and increased tortuosity) in the nail as compared with the hoof

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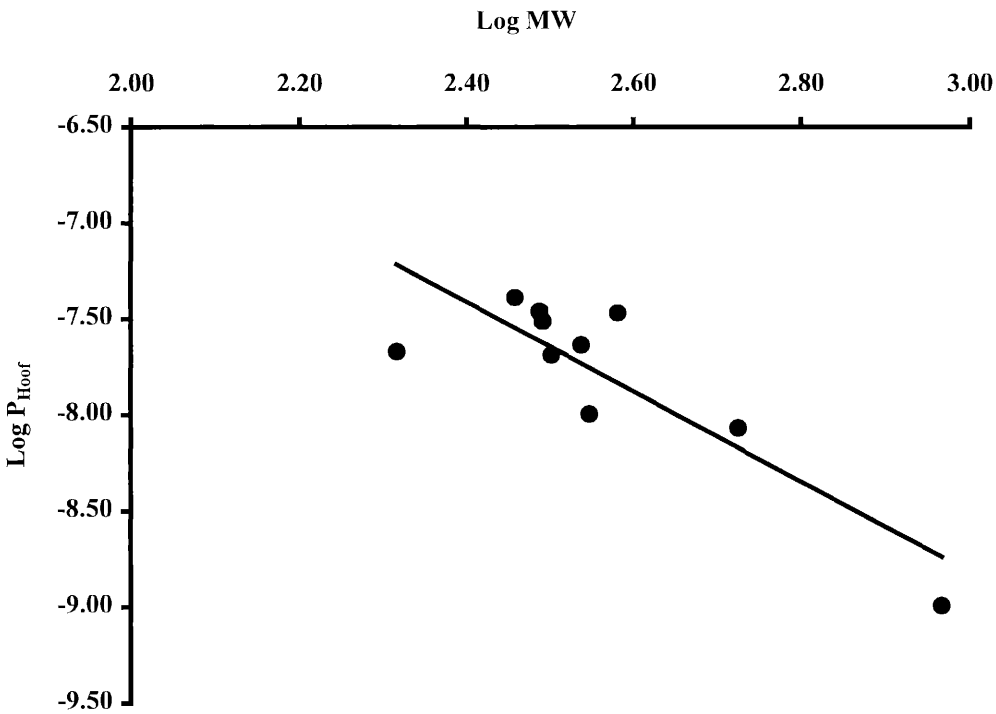


Figure 3. Relationship between the logarithm of molecular weight (MW) of various antimycotics and the logarithm of their permeability coefficients through a bovine hoof membrane (P_{Hoof}). Adapted from reference 37.

membrane, and possibly due to distinct differences between the two keratinous tissues. However, due to the limited availability of nails and the similarity between nails and hoof membranes (both act as hydrophilic gel membranes), the authors suggested that the hoof membrane may serve as an appropriate *in vitro* model to predict nail plate permeabilities. The authors derived the following empirical equation to relate permeability coefficients through the nail and hoof membrane for different drugs:

$$\log P = 3.723 + 1.751 \log P_{Hoof} \quad (1)$$

where P represents the permeability coefficient through the nail plate, and P_{Hoof} represents the permeability coefficient through the hoof membrane. Thus, if P_{Hoof} values are experimentally determined, P values can be calculated. However, due to inherent differences between the two keratinous membranes, caution should be exercised in extrapolating data from the hoof membrane to the human nail.

As stated earlier, the human nail plate is made of dorsal, intermediate, and ventral layers. The *in vitro* permeation of a water-soluble drug (5-fluorouracil) (5-FU) and a water-insoluble drug (flurbiprofen) (FB) through the different layers of the nail plate, using a modified side-by-side diffusion cell, has been reported (38). The thickness ratio of each layer (dorsal:intermediate:ventral) was assessed to be 3:5:2. The dorsal-filed, ventral-filed, and dorsal-and-ventral-filed layers of the nail plate were prepared by filing fingernail clippings with sandpaper to a known thickness. The drug concentrations were measured by HPLC. In this study, it was found that lipids are predominantly present in

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the dorsal and ventral layers. Permeation fluxes for both model compounds were found to follow the rank order: dorsal-and-ventral-filed nail plate > dorsal-filed nail plate > ventral-filed nail plate. The permeation parameters obtained from this study are shown in Table II. The permeability coefficient of 5-FU was found to be highest in the intermediate layer, while that for FB was highest in the ventral layer. The diffusion coefficients for both model compounds were lowest in the dorsal layer. The concentrations of the drugs in each layer corresponded to their water solubility and flux. The results from this study indicate that the upper dorsal layer of the nail plate constitutes the main barrier to the diffusion of drugs. This finding contradicts the earlier study using shaved nails (35), where the dorsal layer was thought not to be the main diffusional barrier. This discrepancy could possibly be due to the difference in methodology used by the researchers. The latest study with 5-FU and FB (38) appears to have carefully controlled conditions, wherein the thickness of each layer of the nail is precisely measured and differences in permeability properties of each layer have been quantitatively demonstrated.

EFFECT OF pH

Various studies have been conducted to assess the effect of pH on nail permeability using ionizable drugs. In order to determine whether vehicle pH affected nail plate permeability, the *in vitro* penetration of an antifungal agent, miconazole (radiolabeled), through the nail was studied at various pH values from 3.1 to 8.2. Miconazole, a weak base with a pKa of 6.65, was thus present in varying degrees of ionization at different pH values. The results of this study indicated that the flux of miconazole was nearly invariant at low and high pH conditions, suggesting that the degree of ionization had little effect on nail permeability. Thus, it was concluded that for this drug, water solubility could be increased by lowering the pH of the formulation without compromising the permeability characteristics (39).

Although the study described above suggests that pH has little effect on nail permeability, other investigations have shown that pH is indeed a factor in altering the nail permeability characteristics of compounds. Soong (35) investigated the effect of pH on the permeation of benzoic acid through the nail plate. In these experiments, the donor contained saturated solutions of the permeant (by maintaining an excess of drug) at various pH conditions. The pH of the receptor compartment corresponded to that of the donor. It was found that as the pH increased from 2.0 to 8.5, the permeability coefficient of benzoic acid decreased by 95.5% and the lag time increased appreciably. The highest permeability coefficient and shortest lag times were observed at the lowest pH (2.0) when benzoic acid (a weak acid) was undissociated. Hence, this study shows that uncharged molecules preferentially permeate the nail barrier. Mertin and Lippold (36) also investigated the effect of pH on nail permeability. Their study involved the evaluation of permeation of benzoic acid (a weak acid) and pyridine (a weak base) at pH values of 2.0 and 7.4 through a bovine hoof membrane. Thus, benzoic acid is in the dissociated state at pH 7.4, while pyridine is dissociated at pH 2.0. Figure 4 shows the change in permeability coefficient with change in pH for these two ionizable drugs. Figure 4 indicates that dissociation of the therapeutic agent hinders its permeation (as reflected by a decreased permeability coefficient). Since keratin is a protein with an isoelectric point of approximately 5, it is positively charged at pH 2.0 and negatively charged at

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Table II
Permeation Parameters of 5-Fluorouracil and Flurbiprofen Through Various Layers of the Human Nail Plate

Layer (thickness ratio)	5-Fluorouracil				Flurbiprofen			
	h (μm)	P ($\times 10^7$) (cm/s)	K_m	D_m ($\times 10^8$) (cm^2/s)	h (μm)	P ($\times 10^6$) (cm/s)	K_m	D_m ($\times 10^8$) (cm^2/s)
Ventral-filed (3:5)	396.0	2.51 ± 0.18	0.53 ± 0.04	1.87	347.2	1.83 ± 0.11	1.07 ± 0.15	5.92
Dorsal-filed (5:2)	346.5	3.07 ± 0.22	0.49 ± 0.01	2.18	303.8	2.76 ± 0.43	1.13 ± 0.24	7.42
Dorsal-and-ventral filed (5)	247.5	7.12 ± 0.65	0.56 ± 0.02	3.13	217.0	3.59 ± 1.28	0.98 ± 0.22	7.96
Full-thickness (3:5:2)	495.0	1.49 ± 0.27	0.54 ± 0.06	1.37	434.0	1.45 ± 0.54	1.47 ± 0.32	4.29

Values represent mean ± S.E.M. (n = 3–5); h is membrane thickness; P is the permeability coefficient; K_m is the membrane/donor vehicle partition coefficient of the drug; D_m is the diffusion coefficient of the drug in the nail membrane.
Adapted from reference 38.

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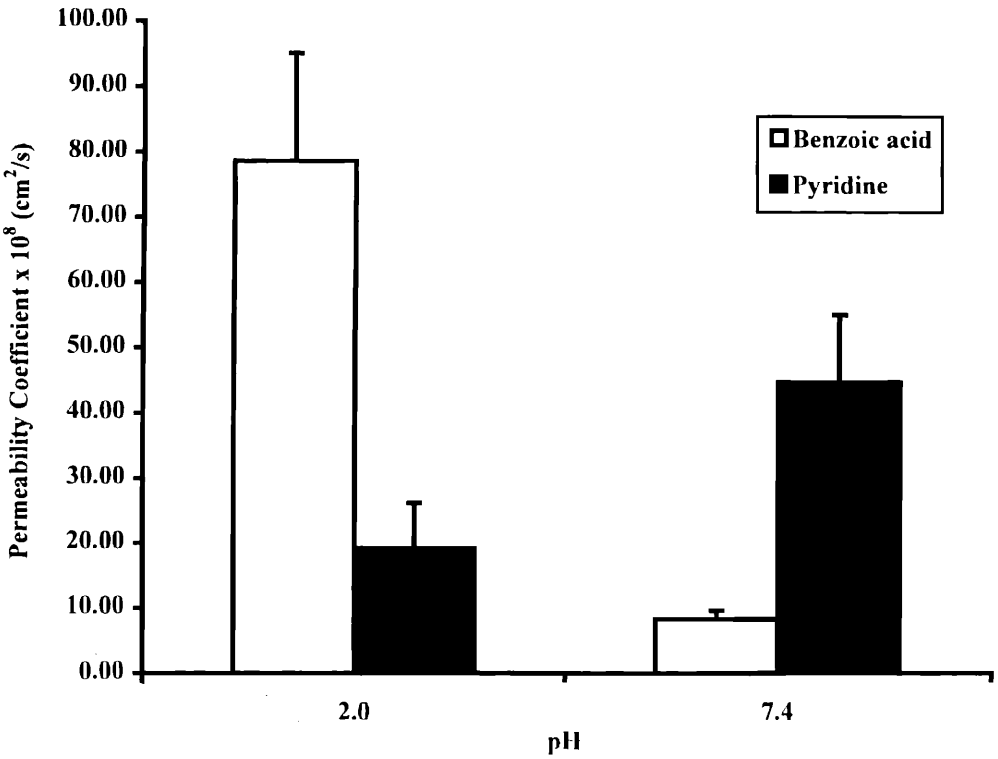


Figure 4. Effect of pH on the permeability coefficients of benzoic acid and pyridine through a bovine hoof membrane. Mean ± S.D., n= 4. Adapted from reference 36.

pH 7.4. The authors suggest that the hindered permeation is a result of the exclusion of the dissociated substance from the membrane due to the Donnan equilibrium effect. Thus, although the studies by Walters *et al.* (39) showed that the permeability of miconazole was essentially pH-independent, the other studies described above suggest that the undissociated drug is preferentially transported through the nail plate.

EFFECT OF VEHICLE COMPOSITION

The penetration of chloramphenicol from an aqueous vehicle (phosphate buffer, pH 7.4) and two lipophilic vehicles (*n*-octanol, and one comprising medium chain triglycerides) through human nail and bovine hoof membranes was studied (40). For the lipophilic vehicles, chloramphenicol was used in a suspended form to ensure maximum thermodynamic activity. Permeation studies were conducted using a modified Franz-type diffusion cell, with temperature maintained at 32°C. The analytical technique used to measure drug concentrations was HPLC. The maximum flux of chloramphenicol (standardized to a barrier thickness of 1000 μm) through the hoof membrane and the nail plate, as a function of vehicle composition, is shown in Table III. It was found that the maximum flux of chloramphenicol through both the human nail plate and the bovine hoof membrane was essentially independent of the nature of the vehicle. Also, from Table III, we see that fluxes through the hoof membrane are at least an order of magnitude higher than those through the nail plate. These results indicate, once again,

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Table III
Maximum Flux of Chloramphenicol (standardized to a barrier thickness of 1000 μm) [J_{max} (1000 μm)] Through a Bovine Hoof Membrane and the Human Nail Plate, as a Function of Vehicle Composition

Vehicle	Maximum flux for chloramphenicol J_{max} (1000 μm) (× 10^{6}) (mg/cm^2/s)	
	Hoof membrane	Human nail plate
Phosphate buffer, pH 7.4	4.07 ± 1.18	0.821 ± 0.211
n-Octanol	3.40 ± 0.68	0.913 ± 0.063
Medium chain triglycerides	4.06 ± 1.00	n.d.[1]

Values represent mean ± S.D. (n = 3–4).
[1] Not determined.
Adapted from reference 40.

that caution must be exercised in interpreting data from other keratinous membranes when information on human nail permeability is required. In the same study, a nail lacquer composed of Eudragit RL [quarternary poly (methyl methacrylate)] and varying chloramphenicol concentrations was investigated. It was found that the lacquer allowed the formation of a supersaturated layer of the drug on the nail plate/hoof membrane and that this resulted in relatively large drug fluxes, particularly with high drug concentrations (up to 31%). Thus, a lacquer-type formulation appears to be especially suitable for topical treatment of nail infections, as it allows sufficient adherence of the formulation to the nail plate.

In another study, it was reported that the absorption of amorolfine through the human nail was affected by the composition of the lacquer in which it was present (41). In this study, the permeation rate of 5% amorolfine (radiolabeled) from an ethanol or methylene chloride lacquer was investigated. The fluxes were found to be higher from the methylene chloride lacquer than from the ethanolic vehicle, with peak fluxes approaching 100 ng/cm^2/h. Also, drug uptake by nails (measured by soaking nail pieces in the test formulation for 48 h) was found to be 2.9 ± 0.6 μg/g nail for the methylene chloride lacquer, as opposed to 1.2 ± 0.4 μg/g nail for the ethanol lacquer. However, the topical efficacy of these amorolfine lacquers in the treatment of OM was not reported. Thus, although the composition of the vehicle may impact the rate of permeation of an antifungal drug and its uptake by nail, topical therapy is not yet effective enough to treat nail disease.

PENETRATION ENHANCEMENT

The limited success of topical therapy is due to the poor penetration rates of the active, which results in prolonged treatment periods. Thus, there is a need to enhance penetration rates with a view towards achieving significant drug concentrations at the site of infection and reducing the duration of treatment. An efficacy coefficient, *E*, was recently introduced (37) to predict the topical effectiveness of an antimycotic formulation. This efficacy coefficient (which should be maximum for high therapeutic effectiveness) takes into account the fact that both the maximum flux and the antifungal potency (expressed as minimum inhibitory concentration) play a role in determining the

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efficacy of a topical antifungal treatment. This coefficient was defined as shown in equation 2:

$$E = J_{max}/MIC \quad (2)$$

where J_{max} represents maximum flux, and *MIC* is the minimum inhibitory concentration of the antimycotic drug. Thus, enhanced penetration (high J_{max}) of a potent antifungal (low *MIC*) would result in an efficacious topical formulation for treatment of OM.

In an early study, the effect of petrolatum, ethylene glycol monomethyl ether (EGME), dimethylacetamide, and dimethyl sulfoxide (DMSO) on nail penetration, in ten subjects, was investigated. A fluorescent dye was used as a marker, and depth of penetration was judged by scraping the nail plate with a knife. Penetration was found to be insignificant with petrolatum and dimethylacetamide. In the case of DMSO and EGME, a maximum penetration of about one fourth the depth of the nail plate was achieved (42). In another study, the conventionally accepted skin penetration enhancer, DMSO, was shown to retard the permeation rate of methanol and hexanol across the nail plate. Isopropanol was found to reduce the rate of nail permeation of hexanol, while it had minimal effect on the permeability of methanol (43). Skin penetration enhancers, such as DMSO, are thought to interact with the lipid domains of the stratum corneum by increasing the fluidity or increasing the partitioning of the drug into it. Due to the low levels of lipid in the nail and the possibly less developed lipid pathways, skin penetration enhancers may be ineffective in accelerating nail penetration (5).

Soong (35) demonstrated that treatment of the nail with agents such as dithiothreitol (containing sulfhydryl groups) resulted in enhanced permeation for various model compounds (acetic acid, benzoic acid, and suprofen). The effect of treatment of the nail with dithiothreitol on the lag times and diffusion coefficients of the three drugs is shown in Table IV. As seen in Table IV, for all three compounds investigated, dithiothreitol treatment resulted in a reduction in lag times and an increase in diffusion coefficients. The greatest effect was observed with the compound of highest molecular weight (suprofen). The enhanced permeation for all the model compounds was attributed to the reduction of the disulfide linkages in the keratin matrix of the nail plate by dithiothreitol and, thus, an increased diffusivity within the nail for the drug. The author suggests that such chemical treatment would be particularly useful in enhancing the permeability of large, charged molecules, which would otherwise have extremely poor nail penetration rates.

Table IV
Effect of Treatment of Nails With Dithiothreitol on the Lag Times (t_L) and Diffusion Coefficients (D) of Various Drugs, Shown as the Ratio of the Parameters of the Treated (t) to the Untreated (u) Nails

		Permeation parameter of treated nail (t)/ permeation parameter of untreated nail (u)	
Model compound	Molecular weight	Lag time $t_L(t)/t_L(u)$	Diffusion coefficient $D(t)/D(u)$
Acetic acid	60	0.62	8.3
Benzoic acid	122	0.47	11
Suprofen	260	0.11	45

Adapted from reference 35.

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The influence of keratolytic agents (urea, salicylic acid, and papain) on the permeation of imidazole antimycotics (miconazole nitrate, ketoconazole, and itraconazole) through the human nail was studied *in vitro* (44). The experiments were carried out using side-by-side diffusion cells, and the donor and receptor solution were composed of a 60:40 ethanol:water mixture. To assay the drug content, the samples were reacted with bromocresol green, and the complex between the imidazole moiety and the dye was measured spectrophotometrically. It was reported that ethanol (used as a co-solvent) did not promote the passage of any of the antimycotics through the nail over a period of 60 days. Scanning electron microscopy studies revealed that all the keratolytic agents modified the normal scaly surface of the nails (as evidenced by a more fractured scaly surface). However, pretreatment of the nails with salicylic acid alone (20% for ten days), or application of the drug in a 40% urea solution, was not effective in enhancing nail permeation. Only the combined effect of pretreatment with papain (15% for one day) and salicylic acid (20% for ten days) was effective. The authors postulated that the keratolytic action of papain in conjunction with salicylic acid results in the formation of pores in the nail, thereby creating transport channels for the drug to permeate.

Novel ways of using chemical enhancers to directly interact with sites on nail keratin were recently reported (5,45). A schematic diagram of the bonds in nail keratin, which represent potential interaction sites for nail penetration enhancers, is depicted in Figure 5. In the patented innovation of Sun *et al.* (45), N-acetyl-l-cysteine (AC) and urea were

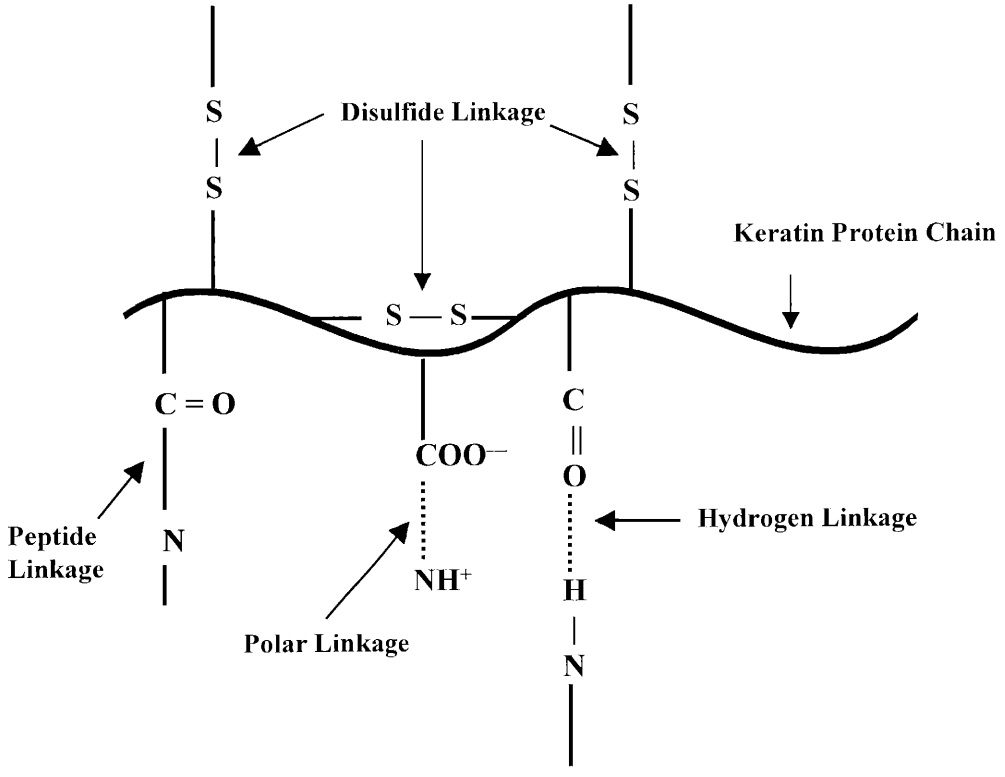


Figure 5. Schematic representation of the bonds in nail keratin that represent potential interaction sites for nail penetration enhancers. Adapted from reference 5.

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used to enhance penetration of the antifungal drugs itraconazole (1%) and miconazole nitrate (2%) through the human nail. AC and other sulfhydrl (–SH)-containing amino acid derivatives were considered to be responsible for cleaving the disulfide linkages (–S–S–) in the keratin matrix, whereas urea was added to break the hydrogen bonds and thus facilitate the cleavage of the disulfide linkages. The reaction sequence between the cystine linkages in keratin and cysteine (containing the –SH group) is shown in Figure 6.

The enhanced penetration of the antifungals into the nail was demonstrated by nail swelling tests and drug partitioning tests (5,45). The swelling test was used as an indicator of rate and extent of drug uptake by the nail, and the partitioning test was used as a measure of drug migration into the nail. These tests were performed by immersing nail clippings in the test formulation at 32°C for two days, rinsing off the adherent formulation after this period, examining the weight gain, and analyzing for drug content by HPLC (after digestion of the nails). The results from the swelling and partitioning studies for 1% itraconazole formulations containing various cysteine derivatives (5%) and urea (10%) are shown in Table V. In addition to the cysteine derivatives and urea, the formulations tested contained salicylic acid (5%), and the vehicle comprised a mixture of propylene carbonate, propylene glycol, and water. The results in Table V are expressed as swelling and partitioning enhancement factors. The swelling enhancement factor was defined as the ratio of percent weight gain of the test nail sample (with enhancer) to that of the control nail sample (without enhancer). Similarly, the partitioning enhancement factor was defined as the ratio of drug concentration in the test nail sample to that in the control nail sample. Table V shows that a promising result was seen for the formulation containing 1% itraconazole, 5% AC, and 10% urea, which resulted in significant nail swelling (threefold greater than control) and drug uptake into the nail (93.6-fold higher than the control without enhancer).

In the same set of studies (45), the effect of enhancers (AC and urea) on the *in vitro* nail permeation of itraconazole and miconazole nitrate was demonstrated. The permeation studies were conducted using nail diffusion cells of area 0.1202 cm^2, wherein the nail separated the donor and receptor compartment. To simulate actual use conditions, the donor chamber was re-dosed with fresh drug formulation at predetermined times (after removing the formulation from a previous application). The receptor fluid used was 20% aqueous hydroxypropyl-β-cyclodextrin, to ensure that sink conditions were maintained. The amount of drug permeated (in the receptor) and the amount of drug in the nail, as

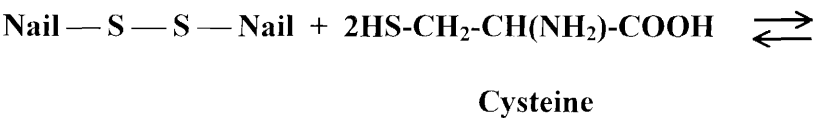


Figure 6. Chemical reaction sequence between nail keratin and sulfhydryl containing amino acid (cysteine). Adapted from reference 5.

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Table V
Nail Permeation Enhancement of 1% Itraconzole Formulations Containing Various Cysteine Derivatives (5%) and Urea (10%), Expressed as Swelling and Partitioning Enhancement Factors

Cysteine derivative (5%) (enhancer)	Swelling enhancement factor[1]	Partitioning enhancement factor[2]
Control (no enhancer)	1	1
N-acetyl-l-cysteine	3.18	93.6
l-Cysteine	4.57	105.0
dl-Homocysteine	2.04	23.5
Cysteamine	3.03	56.5
l-Cysteine methyl ester	2.09	30.7
l-Cysteine ethyl ester	1.82	26.2

[1] Swelling enhancement factor = (% weight gain of the test nail sample)/(% weight gain of the control nail sample).
[2] Partitioning enhancement factor = (drug concentration in the test nail sample)/(drug concentration in the control nail sample).
Adapted from reference 45.

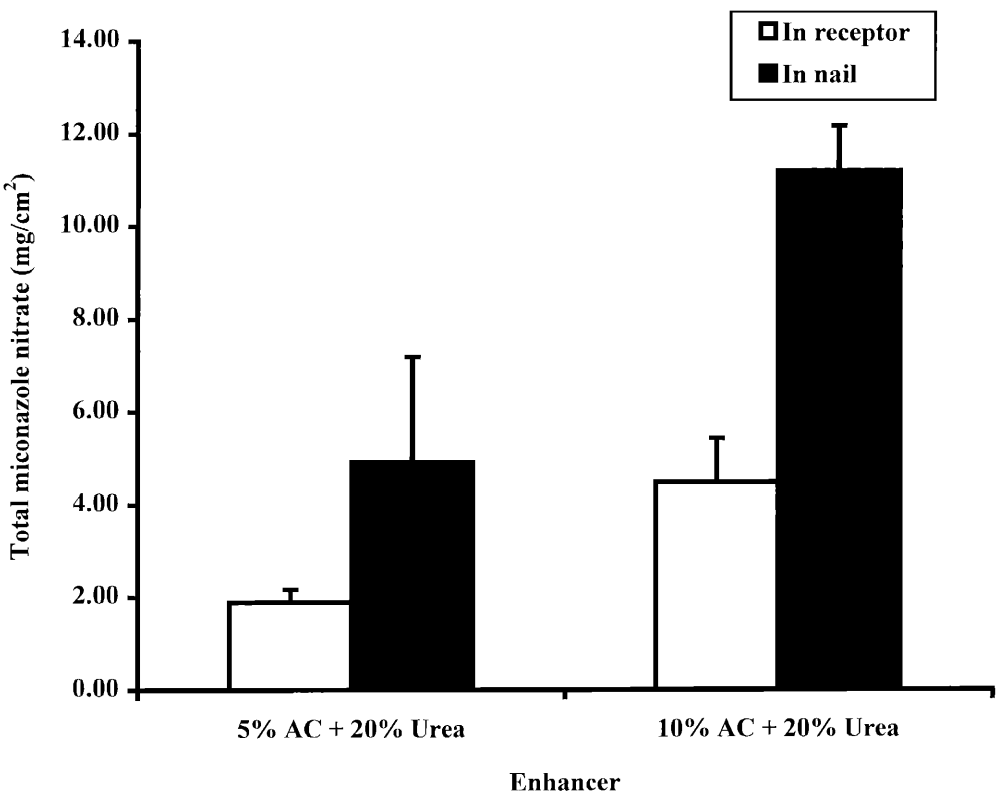


Figure 7. Amount of miconazole nitrate penetrated into and through human nails by treatment with 5% or 10% N-acetyl cysteine and 20% urea for three weeks. Mean ± S.D. Adapted from reference 45.

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a function of AC concentration, are shown in Figure 7. The results depicted in Figure 7 are for 2% miconazole nitrate formulations containing 20% urea and varying amounts of AC, with the donor being replenished every three days. Replenishing the donor compartment at 3- or 7-day intervals did not produce a change in the permeation profiles for miconazole. The total duration of the study was three weeks. From Figure 7, it is seen that doubling the AC concentration from 5% to 10% results in a 2–2.5-fold increase in the amount of drug permeating through the nail, as well as into it. By dividing the amount of drug in the nail (mg/cm^2) by the nail thickness (cm), these researchers calculated miconazole or itraconazole concentrations in the nail (mg/cm^3) from formulations containing AC and urea. It was reported that the drug concentrations measured in the nail exceeded the MICs of these antifungal compounds.

Kobayashi *et al.* (46) also reported the effect of various enhancers on the *in vitro* nail permeation of 5-fluorouracil (5-FU) (water-soluble) and tolnaftate (TN) (water-insoluble) from aqueous and lipophilic vehicles. Analysis of the drugs was carried out by HPLC. Aqueous solvent systems containing urea, sodium salicylate, N-acetyl-l-cysteine (AC), 2-mercaptoethanol (ME), or menthol were used, as well as lipophilic systems containing lactic acid, AC, or ME. Nail weights and stresses (measured using a rheometer) as a result of these treatments were also reported. Nail weights increased and stresses decreased in water-containing solvent systems. The most dramatic effect on nail weights and stresses was seen with aqueous systems containing AC or ME. No significant changes in nail swelling and softening properties were observed with the lipophilic systems. Thus, the authors state that the water content of the nail may relate to its physicochemical properties. Permeation studies using 5-FU and TN were carried out in modified side-by-side diffusion cells (area = 0.049 cm^2), with temperature maintained at 37°C. Fluxes of 5-FU were 13 and 16 times higher for aqueous vehicles containing AC or ME than for the control vehicle (water), respectively. Lipophilic vehicles with AC or ME resulted in a 6.7 and 8.4 times increase in flux for 5-FU, as compared with the control. For TN, the fluxes were only detectable from systems containing AC or ME. The change in physicochemical properties of the nail (weights and stresses) and the increase in drug permeation observed with AC and ME suggest that these two compounds may be effective penetration enhancers for the nail. The authors postulate that the increased permeation results because of a reduction in the keratin disulfide linkages in the nail by the action of AC or ME.

In the same study (46), the effect of AC concentration and reversibility of penetration enhancement by AC was assessed. For this purpose, the permeation of 5-FU in a control vehicle (water) was monitored over a period of seven days (step 1), followed by a washout period of a day. Subsequently, the same nails were treated with 5-FU in vehicles containing AC in concentrations of 0.1–10% for another week (step 2), and the washout procedure (one day) was repeated. Finally, the permeation of 5-FU from the control vehicle (water) was again measured for a week (step 3). Flux ratios (step 2/step 1) were used to investigate the effect of AC concentration, whereas flux ratios (step 3/step 1) were used to assess the reversibility of the treatment. Flux ratios (step 2/step 1) increased with increasing AC concentration. Moreover, flux ratios (step 3/step 1) were very similar to the step 2/step 1 values, indicating low reversibility of the process. The authors speculated that the nail permeability barrier may require more time to recover to its original state.

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TOPICAL TREATMENT OF ONYCHOMYCOSIS: INVESTIGATIONAL STUDIES

Topical medication for OM has remained a secondary form of treatment due to the extremely low permeability of the nail plate to antifungal drugs. Thus, these actives are unable to achieve significant concentrations at the site of infection in order to be therapeutically effective. At best, topical medication is effective in the case of the more superficial forms of OM. Despite the challenges associated with topical therapy, direct application of an efficacious antifungal product to the nail appears to be most desirable, since it is noninvasive and free of systemic side effects.

Some effort has been made in the last two decades in improving the efficacy of topical products intended to treat OM. Farber and South (47) first reported the use of urea for nonsurgical nail removal. Following this, it was reported that a 20% urea and 10% salicylic acid preparation was successful in achieving painless, nonsurgical avulsion of nails after a two-week occlusive application (48). Atraumatic nail avulsion using 50% potassium iodide or 40% urea has been reported to be effective without damage to the nail organ (49). Several investigational treatments now include the use of urea along with an antimycotic agent for the chemical removal of the infected nail. Urea increases the binding capacity of the nail for water, which results in swelling of the keratin matrix, thus simultaneously enhancing penetration of the active. A typical example of an investigational trial is the application of a 1% bifonazole/40% urea ointment until removal of the infected nail, followed by supplementary treatment with a 1% bifonazole cream for four weeks (50). Improvement in the condition of the nails was observed, provided the patients adhered to the treatment protocol. A review of the recent patent literature also shows that urea is commonly used in concentrations as high as 60–70% (possibly to induce noninvasive onycholysis) in conjunction with an antimycotic agent in various types of topical formulations. In cases where such keratolytic agents or other penetration enhancers are absent, very high drug concentrations are used (up to 30%).

Agents capable of interacting with and reducing the disulfide linkages of keratin have recently emerged as a class of enhancers for nail penetration. Van Hoogdalem *et al.* (51) investigated the *in vivo* nail penetration of the antimycotic agent oxiconazole from a 1% w/v lotion, and the potential enhancing effect of 15% acetylcysteine (AC) on this penetration. The topical medication was applied twice daily to the fingernails of six healthy subjects for a period of six weeks, and nail clippings were collected every two weeks over the treatment period and until two weeks after completion of the treatment. The levels of the antifungal drug at various depths in the nail clippings were analyzed by a gas-chromatographic method. The maximum levels of oxiconazole nitrate in the absence of AC were observed in the upper layers (0–50 μm) of the nail clippings and varied from 120–1420 ng/mg. Co-delivery of AC statistically prolonged the mean residence time significantly for the drug in the 51–100 μm layer in the ring fingernails from 3.7–4.9 weeks to 4.1–6.4 weeks, implying increased retention in the nail. Mean drug levels in the upper nail layers also increased in the presence of AC relative to the control, thus suggesting that AC is a potential penetration enhancer for nails. Thus, the use of a reducing agent containing a sulfhydryl group (such as AC) appears to be effective in enhancing penetration of antifungal drugs through the nail.

CONCLUSIONS

Topical management of nail diseases remains a challenge due to the low permeabilities

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of drugs across the nail plate and the extremely long treatment periods required to achieve reasonable drug concentrations in the nail. The highly cross-linked nature of the keratin network makes it difficult for actives to reach the site of infection inside the nail and in the nail bed. The nail lipid content is found to be low. Recent experiments suggest that the dorsal layer of the nail is the main barrier to penetration of actives. The water content and flexibility of the nail are seen to increase after immersion in water, and the nail is found to be much more porous to water than is stratum corneum.

The nail plate has been found to favor the permeation of small molecules and is postulated to behave as a hydrogel barrier in some instances. Some studies indicate that pH affects nail permeation since the unionized form of the drug seems to preferentially permeate, although contradictory results have been reported. It has generally been found that water-soluble drugs permeate the nail in higher quantities, compared with water-insoluble compounds.

Due to the extremely poor permeability of drugs (including antimycotics) across the nail plate, recent research has focused on nail penetration enhancement. A potential method for enhancing drug transport across the nail plate may be to treat the nail with an agent capable of reducing disulfide linkages (–S–S–) in the keratin matrix. Several researchers have reported the utility of N-acetyl-l-cysteine as an effective reducing agent for improving topical penetration of drugs through the nail. Urea has also been reported to have a beneficial effect on nail penetration enhancement.

It is necessary to balance factors such as molecular size, charge, and lipophilicity of the permeant when designing topical products for nails. One must also ensure that the formulation adheres fairly well to the nail plate in order to achieve effective drug concentrations in the nail for a sufficient length of time. Inclusion of compounds that can directly interact with sites on nail keratin can improve drug penetration. Thus, recent studies have allowed to us to better understand the structural and permeability properties of the nail. However, there is still a need for further research so that effective topical treatment for nail disorders can be achieved in the near future.

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