Research Techniques Made Simple: Drug Delivery Techniques, Part 1: Concepts in Transepidermal Penetration and Absorption

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INTRODUCTION

In dermatology, topical therapies are usually the first line and mainstay of treatment for the majority of skin conditions. Most topical preparations are available in a variety of potencies and delivery systems. Practitioners must carefully choose from this vast array based on the potency required, location of intended use, product elegance, and likelihood of patient compliance. Unfortunately, information concerning which preparation is truly best, regarding actual penetration and delivery to the site of action, is not readily available. In general, many practitioners believe that ointments and foams enhance penetration when compared to creams, gels, and powders. However, this is not always the case. Aside from vehicles, there are a variety of chemical and physical enhancement techniques that influence topical penetration. As physician-scientists, dermatologists should be aware of the basic mechanisms involved in topical absorption and should be able to assess whether a preparation is likely to exert its desired effect. In this article, we explore the inherent properties of the epidermis and the physiology of passive diffusion and aim to clarify the definition of the terms "absorption" and "penetration."

KEY POINTS

- The terms "absorption" and "penetration" are often used interchangeably, which is incorrect.
- "Absorption," or "accumulation," refers to the amount of substance that builds up in the skin over a certain period of time whereas "penetration" is the amount of a substance that crosses the skin per unit area per unit time.
- The stratum corneum is the greatest barrier against drug penetration.
- The extracellular lipid composition of the epidermis plays a large role in barrier function.
- There are numerous techniques, both passive and active, to enhance drug penetration through the skin.

ACCUMULATION VERSUS PENETRATION

The terms "absorption," which is the accumulation of drug in the skin, and "penetration," which is a measure of flux/transport across the skin, are often incorrectly used interchangeably. Penetration is quantifiable as the amount of substance that crosses the skin per unit area per unit time. By contrast, "absorption," or "accumulation," refers to the amount of a substance that builds up in the skin over a certain time period. The accumulated substance may remain in the skin or leave the skin to enter the systemic circulation. Whether the substance exerts a biological effect, is inert, is metabolized (and at what rate), and/or is soluble can impact the substance's concentration in the skin at any given time, making the results difficult to compare and reproduce.

There are two general routes through which topically applied preparations can enter the skin: transepidermal (via the stratum corneum) and transappendageal (via appendages such as eccrine ducts and hair follicles). Transepidermal penetration can be either transcellular (through the corneocytes and the lipid lamella) or intercellular (through a complex pathway along the lipid lamella). Generally, it is believed that the penetration of topical drugs occurs primarily through the intercellular route, given the hydrophobic nature of the extracellular space. Although it has traditionally been thought that the transcellular and transappendageal routes contribute only slightly to the overall drug transport, the former is important for small hydrophobic molecules and the latter may in fact be underestimated in certain cases. Transappendageal transport of the follicular type has become a focus of research in the past decade because hair follicles represent an effective reservoir and contain target sites such as stem cells and dendritic cells. The reservoir effect of follicles is profound, measuring up to 10 times larger than that of the surrounding skin (Shah *et al.*, 2014). However, there are two major issues with the follicular pathway. The first is the fact that although some substances can pass deep into hair follicles, none has been able to pass transfollicularly into the surrounding skin or penetrate into the circulation. The second is the lack of a proper skin model other than intact human skin for researching transport through the transfollicular pathway.

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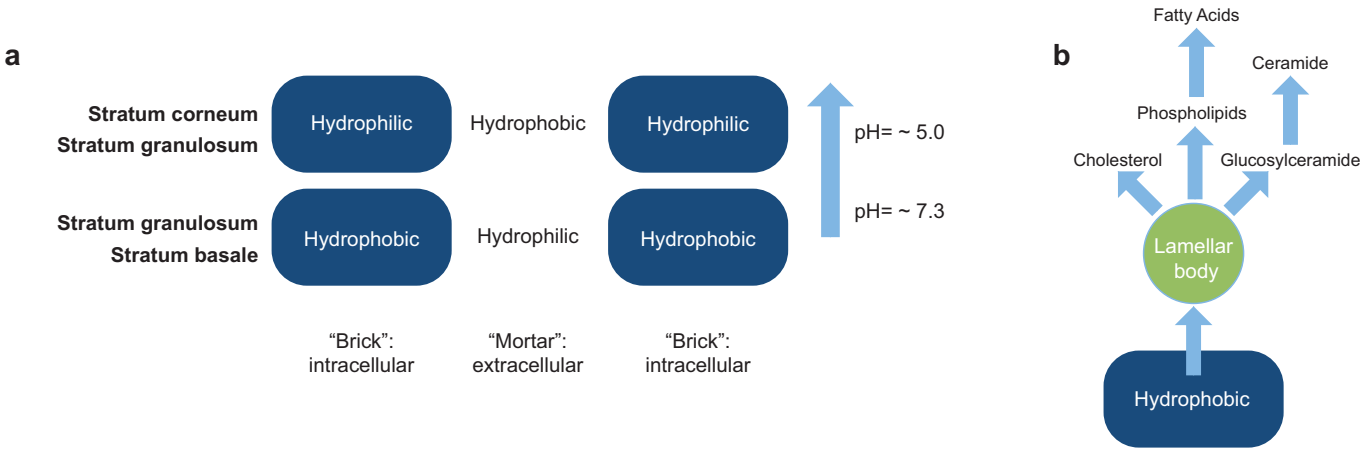

Figure 1. Basic structure of the stratum corneum. (a) "Bricks-and-mortar" model of the epidermis. The extracellular space of the stratum granulosum and stratum corneum exhibits hydrophobic properties due to its lipid-rich composition. This is in contrast to the layers below the stratum granulosum, which exhibit a hydrophilic environment due to the lipid-poor, desmosome-rich composition. pH = ~7.3 in stratum granulosum; pH = ~5.0 in the mid–stratum corneum. (b) Formation of lipid-rich extracellular space. Expulsion of lamellar bodies and conversion of lipids into final end products.

One approach to solving the penetration problem is the use of triggered release mechanisms. This allows the drug to be released independently after it has been delivered deep into the hair follicle. The drug can then pass through the hair follicle and into the surrounding skin. Currently, a number of these release triggers are under investigation and include radiofrequency, ultrasound, light, enzymatic reactions, and pH manipulation (Shah *et al.*, 2014).

BARRIER PROPERTIES OF THE SKIN

The stratum corneum of the stratified epidermis functions as the single greatest barrier against drug penetration (Bouwstra *et al.*, 2001; Jain, 2008). A major component of this barrier is extracellular lipids, which are extruded into the extracellular space as cells transition from the granular layer to the stratum corneum. In conjunction with this lipid-rich extracellular space, limited desquamation of corneocytes provides a homeostatic layer of cells that protects the underlying epidermis. Together, this arrangement of corneocytes in the stratum corneum and the formation of a lipid-rich extracellular space has been coined the "bricks-and-mortar" model (Figure 1) (Bolognia et al., 2012; Bouwstra and Ponec, 2006; Lampe *et al.*, 1983; Hachem *et al.*, 2003; Elias et al., 2006; Shah *et al.*, 2014; Jain, 2008).

The lipid-rich, hydrophobic, extracellular space of the stratum corneum is predominated by ceramides, cholesterol, and free fatty acids (Bouwstra and Ponec, 2006; Shah *et al.*, 2014). This extracellular space is formed by the conversion of lipids extruded from lamellar bodies in the stratum granulosum (i.e., glucosylceramides, sphingomyelin, cholesterol, and phospholipids). The conversion of these precursor lipids into their final end products occurs mainly by the following enzymes, which are also extruded from lamellar bodies: β-glucocerebrosidase, acid sphingomyelinase, secretory phospholipase A2, and proteases. In contrast to the extracellular space of the stratum corneum, the extracellular space in the layers including and between the stratum basale and the stratum granulosum consists of a hydrophilic environment predominated by proteinaceous molecules such as desmosomes (Elias *et al.*, 1977). Because of the importance of the stratum corneum lipids in barrier function, it is likely that there are many enhancers/excipi-

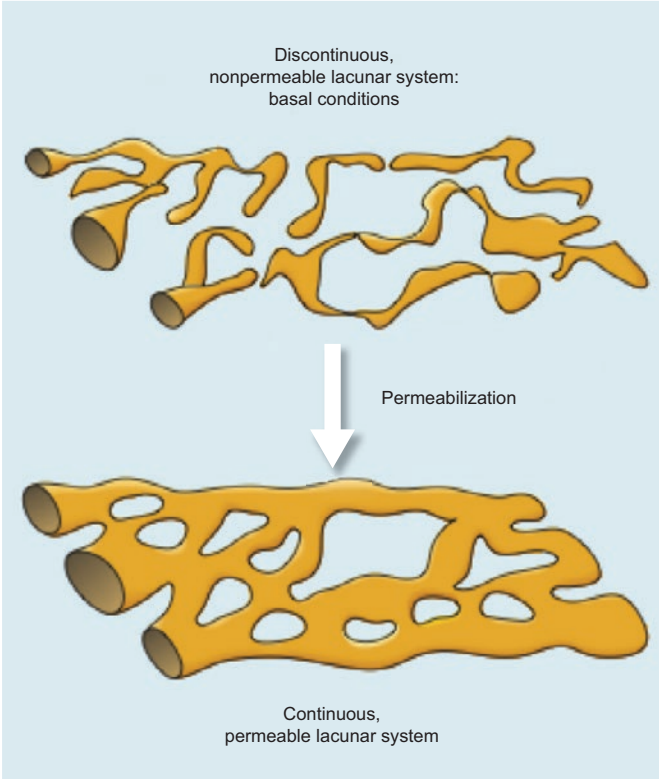

Figure 2. "Pore" pathway within the stratum corneum. Aqueous pores represent discontinuous lacunar domains formed by the degradation of corneodesmosomes. Under certain conditions, such as extensive hydration, occlusion, and sonophoresis, these pores enlarge, extend, and connect, creating a continuous pathway through the stratum corneum. Adapted from Bolognia et al. (2012) with permission from Elsevier.

ents that affect these lipids in some way. Some substances such as DMSO and azone are thought to distort the packing geometry of these intercellular lipids. Oleic acid, azone, and terpenes have been shown to induce discreet domains in the stratum corneum lipids where the excipient is concentrated, resulting in barrier defects. Surfactants such as sodium lauryl sulfate may work by solubilizing stratum corneum lipids (Williams and Barry, 2004).

Table 1. The effect of various vehicles on the permeability of the stratum corneum

Vehicle	Examples	Effect	Permeability
Occlusive dressings	Films, plaster, and transdermal patches	Prevents water loss	↑↑↑
Lipophilic vehicle	Paraffins, oils, fats, waxes, alcohols, esters, silicones	Prevents water loss	↑↑↑
Absorption base	Anhydrous lipids ± emulsifiers	Prevents water loss	↑↑↑
Emulsifying base	Anhydrous lipids ± emulsifiers	Prevents water loss	↑↑↑
Water–oil emulsion	Oily creams (w/o systems)	Retards water loss	↑
Oil–water emulsion	Aqueous creams (w/o systems)	May donate water	↑ (?)
Humectant	Water-soluble bases, glycerol, glycols	Dependent on the concentration of the humectant	Dependent on the concentration of the humectant
Powder	Clays, shake lotions	Aids evaporation	no effect/↑

Adapted from Barry (2001) with permission from Elsevier.

Table 2. The advantages and disadvantages of excipients

Excipient	Advantages	Disadvantages	Other
DMSO	• Effective in both hydrophilic and lipophilic permeants	• High concentrations needed for effect; high concentrations often cause erythema and urticaria • Produces a metabolite that can produce a foul odor on the breath	• Changes keratin confirmation and may distort the packing arrangement of intercellular lipids
Ethanol	• Permeates rapidly through skin	• Concerns about systemic toxicity, especially in the pediatric population	• Mechanism of action may involve evaporative loss of ethanol, leaving behind the drug in a supersaturated state
Pyrrolidones	• Can create reservoirs of a drug and potentially be used for sustained release of a topical drug	• Works better with hydrophilic substances • Can cause erythema and other adverse reactions	• Has been used to enhance transport of mannitol, 5-fluorouracil, β-methasone, hydrocortisone
Fatty acids (oleic acid)	• Effective for a wide range of drugs • Can work with lipophilic and hydrophilic substances • Effective at low concentrations	• Only fatty acids of a certain length and chain figuration have a significant effect on transport	• The *cis*-configuration disturbs lipid packing more and therefore has more potential for transport enhancement
Urea	• Has additional keratolytic properties that can be useful in some disease states	• Has only modest transport enhancement when used alone as an excipient	• Some studies show that synthetic analogues of urea have a greater effect on transport
Essential oils	• Some reports have shown essential oils to have up to a 100-fold increase in permeability coefficient of the target drug • Synergistic effect when combined with propylene glycol	• Effects are often drug specific and not effective for lipophilic drugs	• Used in medicines, fragrance, and flavorings
Phospholipids (nonvesicular)	• Can increase tissue hydration	• Few studies on phospholipids in the nonvesicular form	
Azone	• Low irritation and low toxicity • Low pharmacologic activity • Active at low concentrations • Enhances transport of a wide variety of drugs	• Unknown mechanism of action • Works better for hydrophilic substances than for lipophilic substances	• Thought to disrupt packing arrangement of lipids in lipid bilayers

Adapted from Williams and Barry (2004) with permission from Elsevier.

It has been reported that the conversion of lipids into their final end products is facilitated by acidification of the extracellular space of the stratum granulosum and the stratum corneum. This "acid mantle" is created primarily via two mechanisms (Hachem *et al.*, 2003). The first mechanism involves a non–energy dependent sodium–proton exchanger on the surface of upper epidermal keratinocytes, which pumps protons extracellularly. The second mechanism involves conversion of phospholipids into free fatty acids via phospholipase A2, which provides an acidic environment through the inherently acidic moieties on the free fatty acids. Together, the sodium–proton exchanger and free fatty acids create the necessary pH for optimal activity of β-glucocerebrosidase and acid sphingomyelinase, which convert precursor sphingolipids into ceramides, a very important constituent of the stratum corneum (Hachem *et al.*, 2003).

Just as the "acid mantle" facilitates the formation of a lipid-rich extracellular space to limit drug penetration, so too does it indirectly limit desquamation, further fortifying the barrier function of the stratum corneum (Hachem *et al.*, 2003). Particularly, the low pH of the extracellular space in the upper epidermis limits the degradation of corneodesmosomes, mostly desmoglein 1, by proteases. In contrast, if the pH were higher, protease activity in the extracellular space would increase and desquamation would occur at a more rapid rate. This further illustrates the importance of pH on the barrier function of the skin.

REGIONAL VARIATION

Epidermal permeability changes depending on body site. This knowledge is important when choosing the correct potency and vehicle of a topical preparation, both for efficacy and for avoiding adverse effects. Although most clinicians believe that skin penetration correlates with skin thickness, differences in drug penetration can actually be explained by variations in the number of lamellar membranes (lipid weight %), membrane structure, and/or lipid composition (i.e., sphingomyelin:ceramide ratio) (Bolognia et al., 2012).

PHYSIOLOGY OF PASSIVE TRANSPORT AND ENHANCERS

Transport across the stratum corneum is passive. Fick's law (Jain, 2008), a passive diffusion model, can therefore be used to calculate skin permeation as follows

$$J = \frac{KD}{h}(c_0\text{-}c_1)$$

where J is the flux/unit area, K is the partition coefficient (hydrophilicity), D is diffusity, h is skin thickness, c0 is the concentration of the substance applied, and c1 is the concentration of the substance in the skin. c1 is often set to zero with the assumption that the skin initially contains no amount of the substance being studied. Using this model, inward flux is positively correlated with the hydrophobicity of the topical preparation and the concentration gradient and negatively correlated with the penetration pathway length. In this respect, with additional factors aside, the highest penetration occurs with a hydrophobic drug applied to a thin stratum corneum and no drug already present in the skin. The relationship between flux and concentration gradient can be manipulated via supersaturation by increasing c0, the concentration of substance applied, in Fick's equation.

Although the inherent properties of the stratum corneum and drug are important factors in penetration, the hydration of the epidermis is an additional factor to consider. Although it is not entirely clear by which mechanism water affects skin permeability, it is understood that water typically enhances

QUESTIONS

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For each question, more than one answer may be correct.

1. **The amount of substance crossing the skin per unit area per unit time defines which of the following terms?**
   - A. Accumulation.
   - B. Penetration.
   - C. Absorption.
   - D. Supersaturation.
2. **Which of the following effects is seen with increased pH?**
   - A. Increased desquamation.
   - B. Decreased desquamation.
   - C. Facilitation of sphingomyelinase.
   - D. Increased barrier function.
3. **According to Fick's equation, which of the following will increase flux across the epidermis?**
   - A. High concentration of the substance in the skin before application.
   - B. High partition coefficient.
   - C. High skin thickness.
   - D. Low diffusivity.
4. **Which excipient/enhancer of excipient is thought to work by evaporation and subsequent formation of the drug in a supersaturated state?**
   - A. DMSO.
   - B. Ethanol.
   - C. Azone.
   - D. Urea.
5. **The extracellular space of the stratum corneum is described as which of the following?**
   - A. Hydrophobic.
   - B. Hydrophilic.
   - C. Neutral in polarity.
   - D. Both hydrophobic and hydrophilic.

drug penetration. One hypothesis is that water increases topical drug delivery by exploiting a network of "aqueous pores" (Figure 2). Normally, this network, which consists of lacunae at sites of corneodesmosome degradation, is interrupted (Williams and Barry, 2004). However, under certain conditions, such as extensive hydration, occlusion, and sonophoresis, the lacunae form connections that create a pathway through the stratum corneum (Williams and Barry, 2004; Bolognia *et al.*, 2012). The existence of these pores not only adds to the already numerous transepidermal pathways but also provides future opportunities for drug delivery manipulation (Bolognia *et al.*, 2012). Of particular interest, vehicle characteristics (reviewed in Table 1) can be influential in increasing hydration (Barry, 2001).

Furthermore, the addition of excipients, nonactive additives, can promote penetration. Some of the most studied excipients are water, DMSO, azone, N-methyl-2-pyrrolidone, 2-pyrrolidone, fatty acids, ethanol, propylene glycol, urea, menthol, and essential oils. The advantages and disadvantages of these excipients are outlined in Table 2 (Williams and Barry, 2004).

Finally, another mode of enhancement to consider is the use of nanoparticles, especially given that their use is the most active direction of research in transepidermal drug delivery. In particular, ultrafine nanoparticles ranging from 1 to 100 nm can be utilized to encapsulate drug molecules and enhance penetration. Some common nanoparticles include carbon nanotubes, fullerenes, quantum dots, metals (Ag, Au), metal oxides (TiO2, ZnO, Fe2O3, SiO2), and lipophilic nanoparticles (Delouise, 2012). The size, shape, rigidity, hydrophobicity, and charge of these nanoparticles can be exploited to optimize drug delivery (i.e., to protect labile drugs, control release of drugs, and target drug delivery). However, adverse effects may exist with the use of nanoparticles because they tend to eventually accumulate in peripheral tissue, causing inflammation and damage, no matter their inherent size or chemical properties (Minchin, 2008).

CONCLUSION

As high-volume prescribers of topical drugs, dermatologists should be aware of the factors that influence topical drug efficacy and safety. Specifically, it is important to know what effects varying individual parameters (i.e., excipients, vehicles, etc.) can have on enhancing drug penetration into the epidermis. When presented with a topical drug, a dermatologist can then appropriately decide a drug's true capabilities versus the claims made about the drug. In Part 2 of this Research Techniques Made Simple, we will discuss the techniques used to objectively assess topical bioavailability, giving the reader an even better appreciation of transepidermal drug delivery.

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CONFLICT OF INTEREST

The authors state no conflict of interest.

CME ACCREDITATION

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SUPPLEMENTARY MATERIAL

A PowerPoint slide presentation appropriate for journal club or other teaching exercises is available at http://dx.doi.org/10.1038/jid.2015.343.

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