REVIEW ARTICLE

Percutaneous absorption

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Summary: Clinical effectiveness of topically applied medications depends on the ability of the active ingredient to leave its vehicle and penetrate into the epidermis. The stratum corneum is that layer of the epidermis which functionally is the most important in limiting percutaneous absorption, showing the characteristics of a composite semipermeable membrane. A mathematical expression of transepidermal diffusion may be derived from Fick's Law of mass transport; factors altering the rate of diffusion are discussed.

Résumé: Le médicament topique est efficace à condition que ses ingrédients actifs puissent facilement laisser l'excipient pour pénétrer dans l'épiderme. La couche cornée, démontrant les particularités d'une membrane semiperméable composée, est la couche épidermique la plus importante en ce qui concerne l'absorption percutanée. La diffusion transépidermique peut être traduite sous forme mathématique à partir de la loi de Fick; l'auteur expose certains facteurs reliés à la vitesse de diffusion.

Although a great number of prescriptions for topical preparations are written every day, little thought is given to the mechanisms which underlie liberation of the active ingredient from its vehicle and subsequent absorption in and through the skin. Percutaneous absorption is becoming an increasingly pertinent subject as newer topical formulations, with allegedly more effective bases, appear on the market. Although it may be an oversimplified concept, it remains nevertheless true that the base in which an active ingredient is incorporated will determine, at least in part, its absorption and therefore its therapeutic effect on the skin.

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The barrier

One of the most important functions of the epidermis is to limit free passage of fluid and electrolytes from the highly hydrated underlying tissues to the outside environment; loss of water from the skin is roughly one tenth that which would occur from exposed muscle. This function of water preservation was acquired as an evolutional adaptation, permitting man to survive in a nonaquatic milieu. Reciprocally, the epidermis limits entry of harmful chemicals from the surface into the dermis, and by analogy, absorption of chemicals used in topical medications. It is now well established that the rate-limiting barrier of the epidermis resides in the stratum corneum, or horny layer, and that diffusion of molecules through this layer is purely passive. As such, this process does not require expenditure of metabolic energy; in fact, dead epidermis is as effective a barrier as live tissue. Furthermore, it has been demonstrated that diffusion characteristics through stratum corneum remain the same irrespective of the direction of transport. These observations have allowed extensive *in vitro* studies, leading to a better understanding of the physicochemical dynamics involved in percutaneous absorption.

As mentioned, the stratum corneum is an effective two-way barrier, but is not a complete one and does allow selective transport of some molecules from the surface into the viable epidermis. Percutaneous absorption and therefore pharmacological effectiveness of topically applied medications are based on this property. The rate of diffusion may be negligibly low or relatively high depending on a variety of factors which will be discussed below. Once transport across the stratum corneum has taken place, there is little impediment to transport across the remainder of the epidermis into the dermis.

The stratum corneum

This is the most superficial layer of the epidermis and represents the specialized end-product of keratinization; it is the single most important structure responsible for barrier function in the skin. The relative impermeability of this horny layer is apparent when its permeability constant for water (reflecting ease of diffusion) is compared with that of the dermis: this constant for stratum corneum is 1.1×10^{-7} cm/sec, while the equivalent for dermis is 1.0×10^{-4} cm/sec, a thousandfold increase in rate of diffusion. The stratum corneum is made up of flat, plate-shaped cells 30 μ wide and 0.8 μ deep, closely applied to each other on all sides and interconnected by many desmosomes. These cells, also known as corneocytes, are anucleate and metabolically inert, and contain many longitudinally arranged fibrous tonofilaments in between which an amorphous matrix and remnants of cellular organelles are found. Since the whole layer is 15 to 20 cell layers deep, its total thickness

averages 15 μ. Although all cell layers are involved in barrier function, it appears that the lowermost, more compact layers ("stratum corneum conjunctum") are more impervious. This is not a universally-held opinion, however, and differential permeability may be accounted for by the nature of the diffusing molecules used in various experiments.

An important feature of these cells is the thickness of their membranes. Unlike the plasma membrane of the viable cells of the epidermis (75 to 100 Å), that of cells of the stratum corneum measures approximately 200 Å. However, it retains the structural features of a biomembrane as described by Singer, being composed of a viscous lipid bilayer in which globular proteins are interspersed. In certain areas these mobile globular proteins extend all the way through the lipid phase; it is postulated that 10 Å pores are formed between such proteins at sites of aggregation, establishing a connection between both sides of the membrane. Although lipids make up only 5% of the chemical composition of total hydrated stratum corneum, they represent the major constituent of the cell membrane and impart to it its distinctive characteristics of semipermeability; removal of lipids from the membrane destroys its barrier properties.

Physiologically, the stratum corneum behaves like a composite membrane: this derives from the fact that the first structure encountered by a diffusing molecule is the plasma membrane of the corneocytes, and since lipids are major constituents of this membrane, it follows that the lipid solubility of this diffusing molecule will be important in determining its entry and transport through the stratum corneum.

The stratum corneum permits a continuous, controlled, transepidermal loss of water by diffusion, about 85 to 170 ml/day under average environmental conditions. In addition 300 to 500 ml/day are lost by invisible sweating, bringing the total daily water loss to 500 to 600 ml/day (0.25 to 1.0 ml/cm^2/hr). This diffusion contributes substantially to sustained hydration of the stratum corneum. However, the relative humidity of ambient air is even more important because the water content of the stratum corneum increases in a curvilinear fashion with increasing humidity, from 10 to 20% at 60% humidity to 70% at 100% humidity. Thus, this layer can absorb approximately six times its own weight in water, and in doing so will increase in thickness from 15 to 45 μ. This property of water-binding is related to increased permeability to diffusing molecules, as will be explained below.

Methods of study

Many experimental methods of study of percutaneous absorption are available, each with its own relative merits; a few representative models are mentioned here. The two-chamber method is a commonly used *in vitro* system designed to measure passage of a substance from one chamber to another through a separating membrane; radioactive materials are often utilized. Measurement of decrease in concentration of a given chemical from a fluid chamber applied directly to the skin is a similar, but *in vivo* technique which has proved less reliable. Measurement of disappearance of weak β-emitting compounds applied to the skin, or measurement of their uptake in the dermis, has allowed fairly accurate quantitative determinations since, in these methods, autoradiography may be used in concert with radioactive pulse-counting. Less specific and less accurate experimental models include various *in vivo* methods in which the end-point is a biologic or pharmacologic effect following application of the test substance to the skin; the effect may be local (e.g. vasoconstriction, sweat gland secretion, etc.) or systemic (e.g. as determined by plasma cortisol levels in the case of topical corticosteroid preparations).

Mathematical expression of transepidermal transport

Transepidermal transport does not escape physicochemical laws, and its expression at the outset follows Fick's Law of mass transport. Fick's Law expresses the fact that the flux — or movement — of a molecule diffusing across a membrane is proportional to the difference in concentration of this molecule on either side of the membrane. Thus,

$$J = Kp \times \Delta Cs$$

where J is flux, Kp the permeability constant, and ΔCs the concentration gradient.

Kp, the permeability constant, is a function of the diffusion constant, Dm, and is inversely related to the length of the diffusion pathway, L, which in this context is synonymous with membrane thickness.

$$Kp = \frac{Dm}{L}$$

The diffusion constant, Dm, is a reflection of the inherent diffusibility and mobility of a molecule in a membrane or, stated otherwise, of its intrinsic ability to break molecular bonds as it crosses a membrane. The reciprocal of Dm describes the resistance of a membrane to transport. Kp, the permeability constant, represents an expanded concept of the diffusion constant, taking into account the length of the diffusion pathway (L). Comparison of Kp and Dm for transport of water through stratum corneum and an erythrocyte will illustrate these notions. Even though Dm for the stratum corneum and an erythrocyte are quite similar (4.2×10^{-10} and 6.5×10^{-9}, respectively), Kp is widely different (1.1×10^{-7} and 1.2×10^{-2}) because membrane thickness (L) is also highly dissimilar (15 μ and 0.008 μ.) As a result, flux (J) is much more rapid through the erythrocyte than through stratum corneum (1 ml vs. 0.00001 ml during the same interval and for a given concentration gradient).

For molecules other than water, and molecules in solution, another factor has to be considered — the partition coefficient, Km. This describes how many solute molecules are available for diffusion through a membrane, and is expressed as the ratio of solubility of a solute in the diffusing membrane and in its solvent. Therefore, the higher the Km for a solute, the greater the ease with which it will leave its solvent, and the more affinity it will show for the diffusing membrane. Thus, incorporating this factor in the definition of the permeability constant,

$$Kp = \frac{Km \times Dm}{L}$$

Pentanol and methanol show comparable diffusion constants (1.33×10^{-9} and 1.85×10^{-9}, respectively), but in aqueous solution pentanol is absorbed better than methanol in stratum corneum because pentanol has a higher partition coefficient than methanol (5 vs. 0.6), showing more solubility in stratum corneum (lipid) than in water. The original equation (Fick's Law) may now be rewritten as follows:

$$J = \frac{Km \times Dm \times \Delta Cs}{L}$$

Although this equation is valid conceptually, it will break down under certain experimental conditions; it is accurate only at low concentrations of the solute and when diffusion has reached a steady state. Also, there are other variables which have to be taken into account when precise data are required, viz frictional forces, temperature and electrical forces between molecules.

Pathways of transport

Most authors agree that there are three possible pathways of transport through the stratum corneum.

The transcellular pathway (through the horny cells) is probably the major one; diffusion proceeding transcellularly is called "bulk diffusion". Lipid-soluble substances first penetrate the

lipid-rich cell membrane and make their way in the cell through the nonaqueous, nonpolar lipid-rich matrix between tonofilaments. Small, polar, water-soluble molecules gain access to the protein fraction of the cell membrane and cross the lipid barrier presumably through small pores in between the protein subunits. Thereafter, diffusion proceeds through waterlogged channels (also described as "pores" of approximately 10 Å by some authors) lined by keratin molecules; these channels enlarge when the stratum corneum is further hydrated, and diffusion is consequently accelerated.

Diffusion through pilosebaceous units and sweat glands has been proposed as another pathway of transport and is referred to as "shunt diffusion". Since the stratum corneum invests only the most superficial parts of these appendages, there should be little hindrance to transport in their deeper portions. Even assuming rapid flux, however, only a relatively small number of diffusing molecules are absorbed, when it is recalled that the openings of these appendages account for only one thousandth to one ten-thousandth of the total surface area of the body. Because of rapid absorption, however, it may be important in the first five minutes of transport, before steady state is reached. At this time the transcellular pathway becomes the predominant one. Shunt diffusion may also be of importance in the percutaneous absorption of molecules with very low permeability constants, such as the more polar steroids, and of other large molecules. The relative unimportance of the shunt diffusion pathway is suggested by the observations that diffusion is not impaired in patients with congenital absence of sweat glands; that palmar skin, with its high density of sweat glands, is highly impermeable to most diffusing molecules; that penetration through hair-bearing rodent skin is quite similar to that through non-hair-bearing areas.

The third pathway is the intercellular route. This pathway may be involved in transport of electrolytes through the skin, since these are extremely insoluble in lipid membranes. Shunt diffusion may serve as an alternative means of absorption. Except for electrolyte transport, the intercellular route appears to have no major role in percutaneous absorption.

Factors altering transport

1. Water: maximum hydration of the epidermis is followed by a fivefold increase in permeability. At 60% humidity, molecules diffusing through intracellular channels encounter water molecules which are tightly bound to keratin molecules ("bound" or "absorbed" water); hydration of the stratum corneum causes aggregation of new layers of water molecules between the tightly bound layers. These new layers are referred to as "bulk water", the molecules of which are held only by weak hydrogen bonds, permitting easier diffusion. Stated in another way, hydration of the stratum corneum "enlarges" the pathways of diffusion, and results in an increase in the permeability constant of both polar and nonpolar molecules.

   Dehydration, paradoxically, also enhances absorption by causing damage to the physical structure of the barrier. When the water content of the stratum corneum falls below 10% the layer becomes brittle and cracks easily. Dehydration is a common aftermath of unfavourable environmental conditions, e.g. low temperature and low ambient humidity.
2. Mass: absorption is proportional to mass or concentration of the molecules applied to the skin up to a critical point, at which time this relationship falls; it follows that there is a limit to the effective concentration of topical preparations.
3. Solubility in lipids has been previously mentioned as a factor conducive to increased permeability.
4. Keratolytics enhance absorption by physically damaging or destroying the stratum corneum barrier. Salicylic acid is a well-known example.
5. Lipid and polar solvents (acetone, alcohol) will result in an increase in permeability after prolonged application, by chemical alteration of the horny layer.
6. Surfactants (e.g. soaps) bring the diffusing substance into closer contact with the stratum corneum. Under normal conditions they have little effect, but after protracted usage may lead to partial barrier breakdown.
7. Dimethyl sulfoxide (DMSO) is a polar, strongly hygroscopic solvent showing solubility in both water and lipids. Application of this substance to the skin results in superhydration of the stratum corneum with subsequent increase in permeability. While having no known effect on plasma membranes, it may cause changes in keratin filaments; its *in vivo* effects are short-lived.
8. Local hyperemia may cause an increase in flux or transport by rapid removal of diffusing molecules as they arrive in the dermis, and therefore by increasing the concentration gradient.
9. Increased temperature increases absorption by increasing molecular motion.
10. Dermatoses: certain dermatoses, notably those exhibiting parakeratosis (e.g. psoriasis and eczema), are accompanied by temporary impairment in barrier function. This explains why topical preparations are more effective during the early, acute phases of these diseases, and are progressively less so as healing takes place and barrier function recovers.
11. Choice of vehicle: since the vehicle (solvent) is one of the factors which determine the partition coefficient, Km, and hence transport of the diffusing solute, the chemical natures of the solute, solvent and membrane constitute basic determinants of percutaneous absorption. The occlusive nature of the vehicle is also critical; occlusion leads to increased hydration and local temperature, which result in increased permeability. A very occlusive vehicle or base may be likened to a polyethylene (Saran Wrap) dressing; up to a hundredfold increase in percutaneous absorption has been achieved with such dressings.
12. Particle size: the smaller the molecule, the faster its transport. This factor is more important than molecular weight, which shows a variable relationship with absorption.
13. Viscosity is inversely related to flux.
14. Polar groups: in general, the presence of polar groups on a molecule impairs its transepidermal transport since electrical charges on the diffusing molecule may interact with those along the diffusion pathway.
15. Electrical impedance: electrical resistance and impedance on the skin reside almost entirely within the stratum corneum. A reduction of impedance is accompanied by a comparable increase in permeability.
16. Regional variations: percutaneous absorption varies markedly in different anatomical areas. If skin from the dorsal or ventral forearm is assigned the arbitrary figure 1 in terms of its permeability, skin from the palm, forehead and scrotum would show the following figures, respectively: 0.83, 6.0 and 42.0. It is well documented that potential irritants are more hazard-

ous when applied to the scrotum than elsewhere.

17. Surface sebum film: although originally this was thought to play a role in barrier function, its relative importance is now recognized as very minor.

As well as being an effective barrier, the stratum corneum may in a sense serve as a reservoir for lipid-soluble molecules. This has been shown to be true for some topical corticosteroids, and may be due to the fact that partition forces favour retention of the lipid-soluble substances in the lipid compartments of the stratum corneum, rather than transport through the more aqueous underlying epidermis.

Having considered some of the many variables involved in percutaneous absorption, it is evident that the ideal "base" is yet to be found. Other factors have to be considered in the choice of a base and have not been discussed above because they do not relate directly to the topic. One such factor — a pertinent one nowadays — is cosmetic acceptability, but this alone is subject matter for another discussion.

I would like to thank Doctor Robert Jackson (Ottawa Civic Hospital) and Doctor Jean-Pierre DesGroseilliers (Ottawa General Hospital) for their assistance in the writing of this paper.

References

1. BADEN HP, GOLDSMITH LA: The structural protein of epidermis. *J Invest Dermatol* 59: 66, 1972
2. BAKER H: The skin as a barrier, in *Textbook of Dermatology*, second ed, edited by ROOK A, WILKINSON DS and EBLING FJG, Oxford, Blackwell, 1972, p 249
3. BANGHAM AD: Models of cell membranes. *Hosp Pract* 8: 79, 1973
4. BETTLEY FR: The epidermal barrier and percutaneous absorption, in *An Introduction to the Biology of the Skin*, first ed, edited by CHAMPION RA, GILLMAN T, ROCK AJ et al, Oxford, Blackwell, 1970, p 342
5. BLANK IH: Penetration of low molecular weight alcohols into skin. I. Effect of concentration of alcohol and type of vehicle. *J Invest Dermatol* 43: 415, 1964
6. BLANK IH, SCHEUPLEIN RJ: Transport into and within the skin. *Br J Dermatol* 81 (suppl) 4: 4, 1969
7. BLANK IH: Transport across epithelial membranes, in *Dermatology in General Medicine*, first ed, edited by FITZPATRICK TB, ARNDT KA, CLARK WH, et al, New York, McGraw, 1971, p 109
8. BLANK IH, SCHEUPLEIN RJ: The epidermal barrier to molecular diffusion. Verbal communication, presented at American Academy of Dermatology Meeting, Miami Beach, Fla, December 1972
9. CHAPMAN D: Lipid dynamics in cell membranes. *Hosp Pract* 8: 79, 1973
10. DANIELLI JF: The bilayer hypothesis of membrane structure. *Ibid*, p 63
11. GOLDMAN MF, KALINOVSKY T, POULSEN BJ: The in vivo penetration of fluocinonide through human skin from different volumes of DMSO. *Br J Dermatol* 85: 457, 1971
12. DESGROSEILLIERS JP, LING GM, BRISSON G, et al: Distribution of ^{3}H-betamethasone-17-valerate after topical application in the domestic pig. *J Invest Dermatol* 53: 270, 1969
13. FELDMANN RJ, MAIBACH HI: Regional variation in percutaneous penetration of ^{14}C cortisol in man. *J Invest Dermatol* 48: 181, 1967
14. KLIGMAN AM, CHRISTOPHERS F: Preparation of isolated sheets of stratum corneum. *Arch Dermatol* 88: 702, 1963
15. LIVINGOOD CS, HILDEBRAND JF, KEY JS, et al: Studies on the percutaneous absorption of fludrocortisone. *Arch Dermatol* 72: 313, 1955
16. MAIBACH HI, STOUGHTON RB: Topical corticosteroids. *Med Clin North Am* 57: 1253, 1973
17. MARCHESI VT: The structure and orientation of a membrane protein. *Hosp Pract* 8: 76, 1973
18. MIDDLETON JD: Pathways of penetration of electrolytes through stratum corneum. *Br J Dermatol* 81 (suppl 4): 56, 1969
19. ODLAND GF: Permeability properties of the stratum corneum, in *The Skin*. University of Washington School of Medicine, 1971, p 11
20. POLANO MK, PONEC M, SMEENK G, et al: Factors influencing the penetration of corticosteroids through the epidermis, in *Pharmacology and the Skin*, edited by MONTAGNA W, VAN SCOTT EJ and STOUGHTON RB, New York, Appleton, 1972, p 325
21. SARKANY I, HADGRAFT JW: The influence of formulation on topical corticosteroid activity. *Br J Dermatol* 81 (suppl 4): 98, 1969
22. SCHEUPLEIN RJ: Mechanisms of percutaneous absorption: routes of penetration and influence of solubility. *J Invest Dermatol* 45: 334, 1965
23. *Idem:* Bound water in keratin membranes. *Nature (Lond)* 214: 456, 1967
24. *Idem:* Properties of the skin as a membrane, in *Pharmacology and the Skin*, edited by MONTAGNA W, VAN SCOTT EJ and STOUGHTON RB, New York, Appleton, 1972, p 125
25. SINGER SJ, NICOLSON GL: The fluid mosaic model of the structure of cell membranes. *Science* 175: 720, 1972
26. SINGER SJ: Architecture and topography of biologic membranes. *Hosp Pract* 8, 81, 1973
27. STOUGHTON RB, FRITSCH W: The influence of dimethyl sulfoxide on human percutaneous absorption. *Arch Dermatol* 90: 512, 1964
28. TREGEAR RT: *Physical Functions of Skin*. New York, Acad Pr, 1966, p 185
29. VICKERS CHF: Existence of reservoir in the stratum corneum. *Arch Dermatol* 88: 20, 1963
30. WINKELMANN RK: The relationship of the structure of the epidermis to percutaneous absorption. *Br J Dermatol* 81 (suppl 4): 12, 1969
31. WURSTER DE: Some practical applications of percutaneous absorption theory, in *Pharmacology and the Skin*, edited by MONTAGNA W, VAN SCOTT EJ and STOUGHTON RB, New York, Appleton, 1972, p 153