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Membrane Models for Skin Penetration Studies

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I. Introduction

The development of models is a crucial step in the understanding of complex biological processes. Well-designed models may allow isolation and examination of individual component processes and may provide information to support or disclaim hypotheses concerning how key mechanisms and components contribute to the overall system. In the biosciences, models may provide less time-consuming, less expensive, and less morally objectionable alternatives to carrying out experiments on living animals or humans.

The recent interest in drug delivery via the skin and the increased incidence of toxicity due to transdermal absorption of chemicals has encouraged research in the field and has led to a better understanding of skin biology, pharmacology, and chemistry as well as an enhanced comprehension of the mechanisms of percutaneous absorption. It has also created a need for sound structure–activity relationships and reliable model systems that allow the rate and extent of transport of molecules across the skin to be predicted with confidence. With such information available, it would be possible to estimate a molecule's potential toxic or therapeutic availability based solely on its physicochemical properties or on its behavior in the model system. The objective of this review is to summarize the status of investigations into membrane models for

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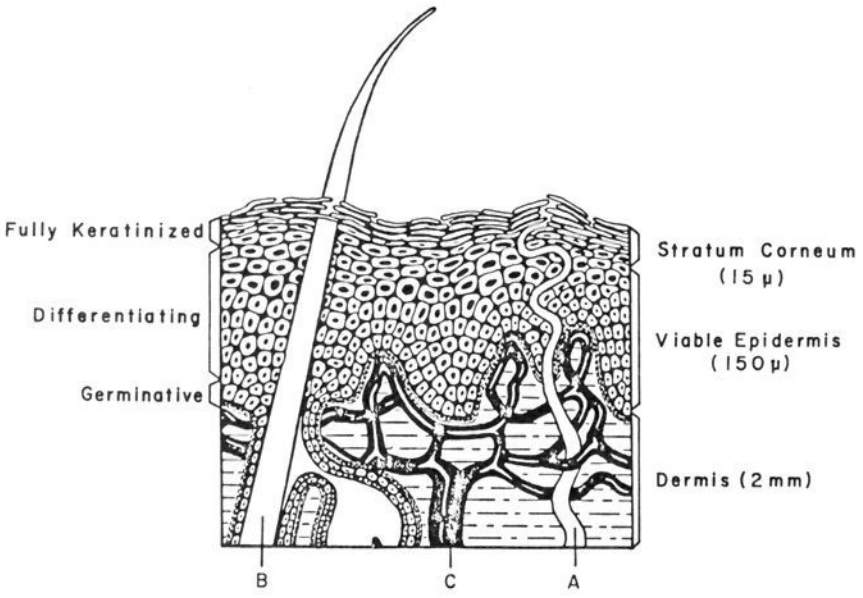

Figure 1. Schematic representation of skin. Percutaneous penetration of a chemical involves transport through and partitioning between the stratum corneum, the viable epidermis, and upper dermis. Hair follicles (B) and sweat glands (A) originate in the dermis and open onto the skin surface. The microvasculature (C) provides access to the systemic circulation for molecules that have penetrated the epidermis.

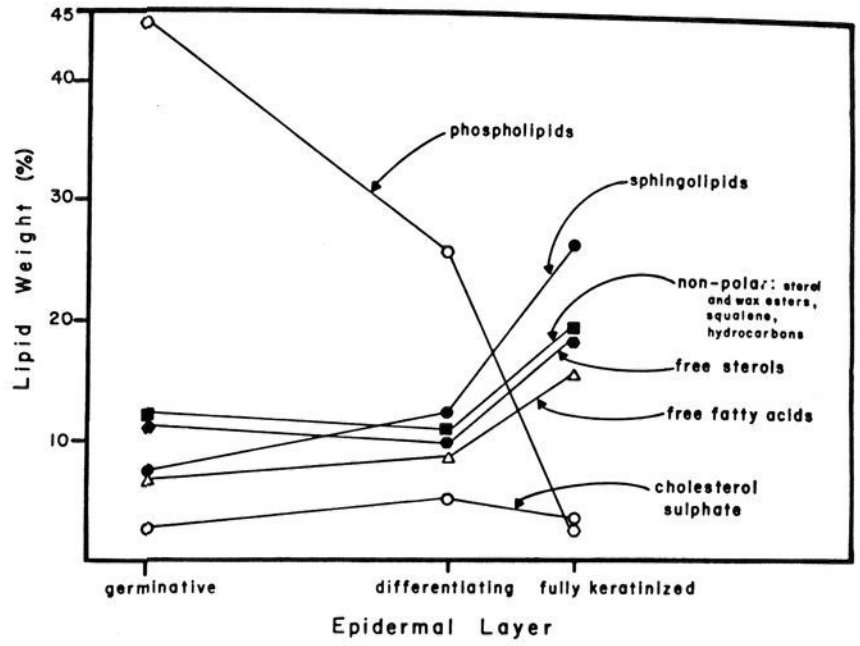

Figure 2. Lipid composition of the various layers of human epidermis. The phospholipid content of the germinative layer is typical of normal living epithelial cells. As the cells differentiate, phospholipids are degraded and other, less polar lipids, especially sphingolipids and sterols, increase and are concentrated between the keratinized cells.

skin penetration and to highlight those avenues that appear worthy of further study.

II. Skin Structure

A good understanding of skin structure and function is critical in evaluating both the relevance and the limitations of any proposed model system. Skin can be structurally viewed as a series of layers, the three major divisions being epidermis, dermis, and subcutaneous fat. Figure 1 is a schematic representation of skin showing the dermis, epidermis, and the major structures that comprise them. The dermis is essentially an acellular collagen-based connective tissue that supports the many blood vessels and nerves of the skin. Hair follicles and sweat glands originate in the dermis and open directly onto the skin surface. The avascular epidermis consists of stacked layers of cells. The living, rapidly proliferating, cells of the inner "viable epidermis" undergo an ordered differentiation process in which they ultimately die and are desquamated. This process involves progressive flattening and elongation of individual cells as well as stratification of the tissue by accumulation of keratin inside the cells. The keratin is dense crystalline protein and is arranged in interconnecting fibers throughout the cell interior. The outermost layer of the epidermis, the stratum corneum, is composed of 10–15 layers of such flat keratin-filled cells, closely packed in a nonpolar lipid matrix. The major lipid components of this intercellular matrix have been recently characterized[1] and have been found to be quite different from the lipids present in living epithelial tissue, including the underlying viable epidermis. Lampe et al. have quantified these lipids in the germinative, differentiating, and fully keratinized epidermis and have found a progressive depletion of phospholipid coupled with increasing amounts of neutral lipids, especially sterols, and sphingolipids.[2] These changes in lipid composition are likely to be involved in modulation of skin permeability and possibly in the regulation of stratum corneum cohesion and desquamation.[3] Figure 2 shows the relative amounts of the major lipid constituents in human epidermis and how they change during epidermal differentiation. It has been proposed that, based on recent electron microscopic and X-ray diffraction results, despite the low phospholipid content (<5%) of the stratum corneum intercellular matrix, the lipids are indeed arranged physically as multilamellar bilayer sheets.[4–6]

III. Mechanism of Percutaneous Absorption

Transdermal penetration of a chemical involves partitioning between and transport through the stratum corneum, the viable epidermis, and the upper dermis. The dermal blood supply is generally considered to be sufficiently high such that any molecule that has diffused beyond the epidermis will be taken into a capillary and carried away to the systemic circulation. The total resistance to penetration should be the sum of the individual resistances imposed by each layer. Experiments using excised human skin have shown that the overall resistance is characteristic of passive Fickian diffusion[7] and that for a large number of chemicals, diffusion through the stratum corneum is the rate-limiting barrier.[8] (Comprehensive reviews covering percutaneous penetration in more detail include those of Scheuplein and Bronaugh,[9] Barry,[10] and Flynn.[11])

The nature of the stratum corneum diffusional barrier can be further characterized by considering how the physicochemical properties of the penetrant influence transport through the skin. Compounds that are good penetrants have a balance between water solubility and lipid solubility. For a homologous series, penetration is found first to increase with increasing oil/water partition coefficient and then plateau or even decrease for compounds with larger partition coefficients. These relationships were first quantified with a series of straight-chain alcohols by Blank.[12] Correlations have also been found for steroids,[13] phenols,[14,15] and nicotinic acid derivatives.[16] This type of partitioning–permeability behavior is characteristic of diffusion through oil or hydrophobic polymer membranes[17,18] and suggests that the stratum corneum acts essentially as a lipophilic diffusional barrier. However, molecules that are exceptionally hydrophilic or hydrophobic often have permeabilities different from those that would be an-

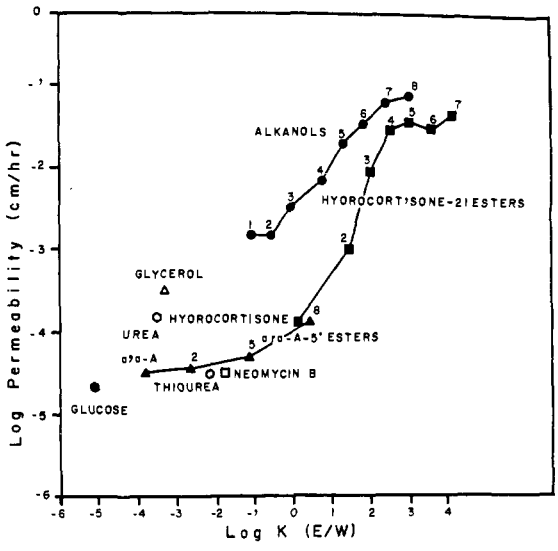

Figure 3. Permeability coefficients of various solutes for their transport across hairless mouse skin plotted as a function of penetrant ether/water partition coefficient (a measure of relative lipophilicity). Three regions of permeability can be distinguished based on permeant lipophilicity (see text for details).

ticipated from the general trends. Very polar molecules tend to be better penetrants than predicted and have permeabilities that depend on molecular size more than on partition coefficient.[19] The evidence suggests that parallel paths exist through the stratum corneum, one hydrophilic (via "aqueous pores", either associated with the hydrated intracellular keratin or through the limited aqueous phase between intercellular lipid bilayers) and one hydrophobic (within the intercellular bilayers themselves), and that the polarity of the penetrant will determine which route will be most important.[11] Flynn and co-workers have measured the permeability coefficients through hairless mouse skin for a diverse group of compounds. These include the homologous alkanols (C_1–C_{10}), hydrocortisone and its 21-alkyl esters, a number of vidarabine 5′ esters, and glucose, glycerol, urea, neomycin, and thiourea.[11] A plot of the calculated permeability coefficients versus the permeant ether/water partition coefficient is shown in Figure 3. Although there are not yet such experimentally standardized data available for human skin, there do seem to be qualitative parallels to the behavior seen with mouse skin. Figure 3 summarizes the results, which support the suggestion that three distinct regimes of permeability exist through the tissue and that the behavior observed depends on the degree of solute polarity. The observed plateau in permeability seen for very nonpolar compounds has been explained in terms of a change in the rate-determining mechanism, from membrane diffusion to aqueous boundary layer control.[11] An alternative argument to explain the change in mechanism invokes the diminished ability of very hydrophobic molecules to partition out of the skin and back into the aqueous receptor fluid (that is, what would be the more aqueous viable epidermis for in vivo penetration).[20]

In addition to considering such structure–penetration relationships, it is important to relate the observed trends to the structural arrangement and chemical composition of the stratum corneum. The keratinocyte–lipid matrix of the stratum corneum has been instructively idealized as a brick and mortar wall.[21] The bricks represent the dead keratin filled cells and the mortar the continuous interstitial lipid phase. Transport through such a matrix can be considered to occur via two possible pathways: (1) a direct path involving repeated partitioning into and out of cells together with diffusion through both inter- and intracellular phases; (2) a more tortuous path in which the molecule diffuses within the intercellular lipid phase only. Although the intercellular space contributes only 10–30% of the total stratum corneum volume,[22] there is increasing evidence to suggest that diffusion within this space is indeed the major mechanism of penetration through the bulk stratum corneum. The evidence includes the following:

(1) If the lipid component of stratum corneum is disrupted by extraction with organic solvents, such as chloroform/methanol, the resistance to solute transport is significantly reduced.[23]

(2) Compounds have been visualized in the intercellular spaces by autoradiographic techniques[24] and, for the penetrant butanol, by a novel in situ precipitation induced by exposure to osmium vapor.[25]

(3) A mathematical analysis of the in vivo penetration of methyl nicotinate supports the intercellular rather than transcellular route.[26]

(4) It has been shown, using differential scanning calorimetry and Fourier transform infrared spectroscopy, that increases in stratum corneum lipid fluidity are accompanied by increases in both water and salicylic acid flux.[27-30]

An important consequence of the existence of a continuous lipid pathway across the stratum corneum is its implication for the use of simple lipid membranes as model skin barriers.

IV. Model Systems

A. Overview

The ultimate "test" of any model system is its ability to yield observations in agreement with the more complicated process that it is intended to mimic. For percutaneous penetration in man, this means in vivo experiments in humans. Unfortunately, these experiments are expensive, and interpretation of the results is further complicated by the high inter- and intraindividual variability often found in the data. Such experiments are certainly precluded when the compound of interest has known or uncharacterized toxicity. Alternatives to in vivo studies in humans include in vivo studies in animals and in vitro experiments using excised skin (human or animal). Correlation between excised human skin and penetration studies performed in vivo on human volunteers has been demonstrated in a limited number of studies.[31-33] Caution must be taken in accepting in vitro data when the compound is likely to be metabolized by the skin. Although metabolic steps that do not require energy (e.g., ester hydrolysis) have been successfully studied in vitro after prolonged periods postexcision,[34] the percutaneous penetration of benzo[*a*]pyrene and related compounds has been shown to be highly dependent on the metabolic viability of the tissue used.[35] Skin metabolism may also be important for steroids and organic nitrates. Human skin for in vitro studies is obtained from cadavers, or following cosmetic or plastic surgical procedures, and is subject to variability in the age, race, anatomical site, and

general health of the donor. Extreme care is necessary in the preparation of human skin membranes. The length and method of skin storage may also introduce variability.[36] Excised animal skin can be defended as a representative and reproducible model in a number of specific cases.[37] The relevance to human data has been shown to depend on both the animal and the specific permeant molecule. Although the use of animal skin can minimize the problem of sample-to-sample variation encountered with human skin (and provides a "natural" membrane that is functionally similar to human skin), it has not eliminated the structural complexity of the tissue. In addition, as with human skin, it remains difficult to make conclusions about mechanisms or specific interactions.

The problems involved in working with real skin make simpler, better characterized, membrane systems appear attractive as models for transdermal absorption. Synthetic membranes will provide the most useful information about the in vivo process when (1) the passive diffusional barrier imposed by the stratum corneum is the major resistance to transport, (2) the molecule of interest is known to be metabolically inert and not specifically bound in viable skin, (3) there is some information (from the penetrant's physicochemical properties) about the probable importance of the possible penetration routes available in vivo, and (4) in vivo experiments of similar design have been or can be performed and correlated with the in vitro results.

Many previous studies using model membranes for skin have been concerned with evaluating the effect of changing vehicle and formulation variables on the observed overall drug availability.[38] (A pharmaceutical formulation is the finished product containing the active drug. The formulation includes a solvent or "vehicle" for the drug such as mineral oil, glycerol, or alcohol and other ingredients that serve to stabilize, emulsify, color, etc. the final product.) Although this will certainly be an area in which model skin membranes will continue to be important in the future, past studies have not always considered the mechanistic relevance of the membrane system employed. In most instances, the membrane acts merely to separate physically the formulation from the receptor phase with the release rate determined by the formulation, not the membrane. This is fine for developing therapeutic drug delivery systems, since it is desirable to rely on the system rather than the skin to control drug input into the body, but such studies do not indicate the utility of the membrane as a model for skin itself. Parenthetically, it may be added that most artificial membranes are more permeable than real skin and, in many cases, therefore, a formulation that controls release through a simulated skin membrane will not determine in vivo absorption.

Solute transport through a membrane that divides two aqueous solutions (typically a "donor" phase that initially contains solute and a "receptor" phase in which solute accumulates once having negotiated the membrane barrier) takes place either by partitioning into and diffusion through a distinct membrane phase or by diffusion through a continuous donor–membrane–receptor phase via channels. Cellulose acetate filtration and all dialysis membranes are examples of such porous barriers. Solutes (and bulk solvent) diffuse through the solution-filled pores, and the polymer provides only a

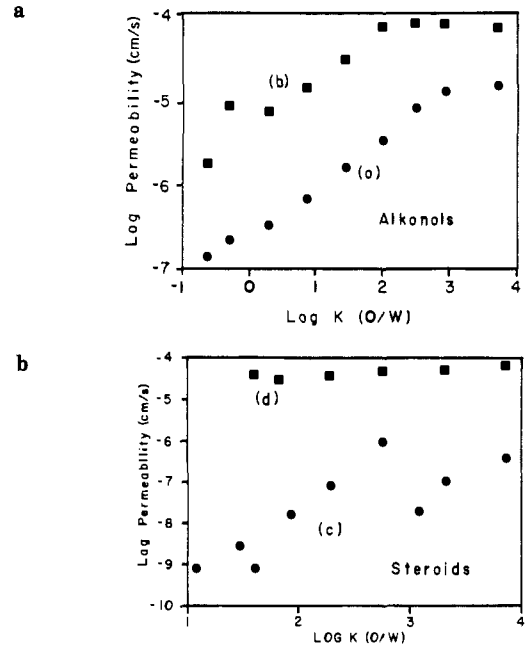

Figure 4. Dependence of penetrant permeability coefficient on partition coefficient (octanol/water): (A) alkanols (C_1–C_{10}) across (a) human epidermis[12] and (b) across silicone rubber membranes;[51] (B) steroids across (c) human epidermis[11] and (d) across cellulose acetate membranes.[39] Silicone rubber exhibits a transport resistance profile which is similar to the epidermal membrane. The shape of both curves is typical of a lipoidal barrier. Cellulose acetate membranes show resistance profiles which are essentially independent of solute lipophilicity and are not representative of transport across intact epidermis.

physical barrier to free movement. When solute transport is via pores, the rate of diffusion is determined essentially by two factors: (a) the probability that a molecule will encounter a pore (concentration) and (b) the tortuosity of the pores (effective diffusional path).[39] Transport is relatively insensitive both to the oil/water partitioning properties of the solute and to molecular size (providing it is much smaller than the size of the pores). Although this mechanism is not representative of diffusion through intact skin, it may be relevant as a model for damaged skin, where the relatively aqueous dermis is the only remaining barrier.[40] Partitioning membranes require solutes to partition into and to diffuse through a phase distinct from that of the donor and receptor solutions. The membrane systems used are generally hydrophobic and include both solids (nonporous polymers, phospholipids below their crystal transition temperature) and liquids (hydrocarbons, long-chain alcohols, isopropyl myristate). Such systems have been used to model drug transport through biological lipid barriers and to correlate the transport rates with the physicochemical properties of the drugs studied. Specific molecular structure, absolute aqueous solubility, and the relative affinity of the molecule for the membrane versus the aqueous phase have been shown to be important determinants of permeability.[17,18] If skin transport is determined by the lipophilic diffusional barrier of the stratum corneum, partitioning-membrane systems can provide mechanistically relevant models. These general observations on partitioning–permeability relationships for different membrane barriers are illustrated in Figure 4 with some specific examples. Figure 4A compares, as a function of solute octanol/water partition coefficient, the

permeability coefficients of a series of 1-alkanols across a simple partitioning membrane (silicone rubber) with the corresponding values across excised human epidermis in vitro. Figure 4B shows a comparable plot for a series of steroids except that the skin permeability coefficients are now compared to those across a cellulose acetate filtration membrane. It seems reasonable to suggest on the basis of these observations that the epidermal barrier for these penetrants is correctly modeled by a lipoidal partitioning membrane.

In a typical experiment, a "transport system" is constructed by dividing two halves of a diffusion cell with the model membrane. Solute (usually in aqueous solution or neat as a thin film) is delivered to the membrane from the "donor" side, passes through the barrier presented to it by the membrane, and finally appears in the opposite "receptor" compartment. Transport is monitored by sampling and analyzing for solute in either the donor or receptor phase as a function of time. Alternatively, the receptor phase may be continuously replenished and analyzed in a flow-through sampling device. When the dose delivered to the membrane is "infinite" (the donor solution contains a large excess of penetrant relative to the amount diffused), there will first be an initial lag period in which the concentration gradient across the membrane is established. After this lag time, solute flux will become constant and can be related to an overall rate constant for the process by solving Fick's first law of diffusion. The general form of the solution is $J = AP\Delta C$, where J is the experimentally measured flux, P is the overall rate constant for transport (or permeability coefficient), A is the area of membrane, and ΔC is the concentration gradient of solute across the barrier. When the membrane is homogeneous and presents the major barrier to transport, the permeability coefficient is given simply by DK/h (D is the solute diffusion coefficient in the membrane, K is the partition coefficient of solute between the membrane and donor/receptor phases (assuming that they are the same), and h is the diffusional path length across the membrane). (Since it is the concentration gradient inside the membrane that provides the driving force for diffusion, the partition coefficient must be included in the permeability expression to correct the concentration of solute measured in the aqueous donor phase to the concentration in the membrane itself.) Diffusion coefficients depend on molecular size, and for the range of solutes usually examined, the difference between diffusion coefficients will be small compared to the corresponding partition coefficients. This makes the partition coefficient the more important of the two in determining permeability. However, the predicted linear dependence of permeability on partition coefficient is not always the observed relationship for transport across a complex membrane such as skin, and, even for the simplest of membranes, deviations from linearity begin to appear outside a certain range of partition coefficient. This can be understood by considering in more detail the physical steps involved in the overall transport process. Even in a simple membrane system, the frictional interaction between the stationary membrane and the stirred solution creates unstirred layers in the solution adjacent to the membrane. In order for a molecule to pass from bulk donor solution to bulk receptor solution, it must (i) diffuse

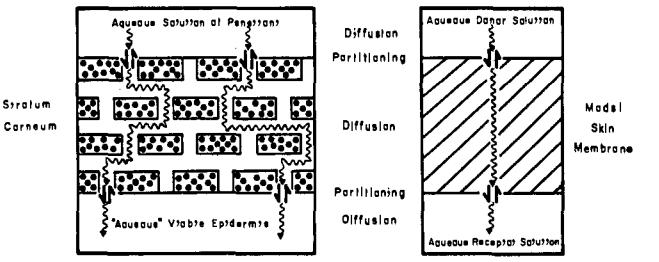

Figure 5. Schematic representation of solute transport through (a) the intercellular lipoidal phase of stratum corneum and (b) an artificial membrane model for skin. Solute transport occurs via a similar sequence of diffusion and partitioning steps.

through the unstirred aqueous layer on the receptor side (the thickness of this layer will depend on the efficiency of stirring and the viscosity of the solution), (ii) adsorb and then partition into the membrane upon reaching the surface of the membrane, (iii) diffuse through the membrane, (iv) partition back out into the aqueous unstirred layer on the receptor side, and (v) diffuse through this aqueous stagnant layer to the bulk receptor phase. Each of these steps presents a resistance (reciprocal permeability) to solute transport, and the total resistance of the barrier will be the sum of these individual components. The steps are shown schematically in Figure 5 for solute transport across skin and across a simple model membrane. This means that transport can progress only as fast as the slowest step in the series. In the classical membrane transport case, this corresponds to diffusion through the membrane and the permeability coefficient will be described by DK/h. There have been, however, a number of transport phenomena reported in which other steps in the overall process are rate limiting.[41] This is also the case for skin transport when the lipophilicity of the penetrant molecule becomes large. There is debate as to whether the limiting step for transport of these highly lipid-soluble compounds is diffusion through the large aqueous stagnant layer imposed by the viable epidermis or is the slow transfer out of the stratum corneum into the aqueous viable tissue.

It can be argued that aqueous donor–membrane–aqueous receptor transport systems may not mimic the normal clinical situation involved in delivering drugs transdermally. Topical dosage forms include traditional ointments and creams, as well as more recently introduced controlled-release devices. Water is seldom the vehicle in these preparations, and even if it were, it is unlikely that a large bulk solution of penetrant could be efficiently maintained on the skin for a period sufficiently long to establish pseudo-steady-state conditions. Experiments using excised skin in contact with water on both sides are subject to the same clinical relevancy question, and in addition, to the question of how the resulting increased hydration of the tissue effects its integrity as a barrier. Better correlation to in vivo absorption profiles have been found (using excised skin) when a "finite dose" of the chemical is applied neat, or in a small amount of organic solvent, to the air-exposed surface of the skin. The dermal side of the tissue is in contact with the receptor phase and samples or a continuous perfusate through this compartment is analyzed for solute (as in a steady-state experiment). The data are generally reported as percent dose absorbed in a certain time period (e.g., 24 or 48 h) or as

maximum flux (i.e., percent of total dose absorbed per hour). It is unfortunate that this methodology has not been adopted in any of the synthetic systems since the form of the data would be similar to that which can be measured in vivo. Nevertheless, steady-state (infinite dose) experiments do yield permeability parameters for both model and excised skin membranes critical for understanding the molecular diffusional processes responsible for the overall barrier behavior. The mathematical complexity involved in solving diffusion equations for the non-steady-state situation (Fick's second law) and the complexity of the biological system make such parameters very difficult to obtain from in vivo experiments.

Of the several membrane systems that have been introduced as potential model barriers for skin, two have been given the most extensive consideration: dimethylpolysiloxane membranes and a number of solid-supported liquid membrane systems. Less well-characterized systems include eggshell membranes, synthetic zeolites, and various multicomponent synthetic membranes.

B. Eggshell Membranes

The eggshell membrane was suggested by Washitake et al.[42] as a possible model for skin because, like the stratum corneum, it consists mainly of keratin. Eggshell membranes were prepared by soaking a whole chicken egg in 0.5 N HCl solution until the outer calcerious shell was dissolved. Part of the membrane was cut away to allow removal of the inner contents, and the remaining membrane was washed and stored in distilled water. Salicylic acid was chosen as a model drug and the permeability through eggshell membranes as well as through cellulose acetate, polyamide (nylon), and eggshell membranes impregnated with isopropyl myristate (IPM) were compared. The IPM-containing membrane was prepared by exchanging (under vacuum) the distilled water for IPM followed by a brief rinsing in water to remove excess IPM. This resulted in partial rehydration of the keratin so that the final membrane contained approximately 50% water and 20% IPM. The release of β-methasone 17-valerate from topical ointments was also measured across the same artificial membranes, and the results were compared to an in vivo assay that assessed the degree of vasoconstriction induced by the steroid once it has penetrated the stratum corneum. The permeability of eggshell membrane alone was found to be independent of pH, in the same way that a cellulose acetate (or any sieving type) membrane would be expected to behave (Figure 6, curve a). It was concluded that solute diffusion took place through water-filled channels in the fibrous protein and that the keratin network is more porous than cellulose acetate. When IPM was incorporated into the keratin network, the permeability behavior changed to that characteristic of a partitioning membrane (Figure 6, curve b). The permeability coefficient was considerably larger than that reported for excised human skin. The order of vasoconstrictor activity found for a series of three ointment formulations was the same as that through all the membranes studied. The observed release profiles in vitro all showed a square root dependence on time. This suggests that the membranes were not sufficiently resistant to be rate limiting with respect to steroid appearance in the receptor phase and that control of the release in each case was due to diffusion out of the ointment.

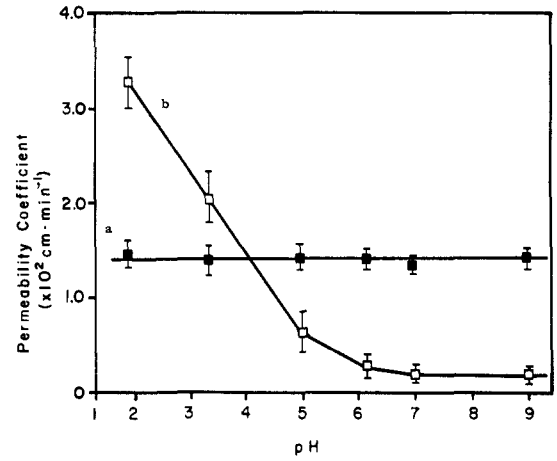

Figure 6. Permeability coefficient as a function of pH for salicylic acid transporting across (■) eggshell membranes and (□) eggshell membranes impregnated with IPM. Without the organic phase, diffusion is through aqueous pores in the keratin network and the permeability coefficient is independent of pH. With the IPM present, partitioning is important and permeability decreases as pH increases when increasing amounts of drug are present as the less lipid soluble ionized salicylate.

In summary, this membrane system contains both hydrated proteinaceous and lipoidal phases known to be present in stratum corneum and is a potentially promising model for skin transport studies. Further characterization of the system using additional penetrants and different organic phases is necessary to reinforce and lend additional weight to the results obtained in this initial study.

C. Composites and Laminates

An approach, which seems sensible considering the multiphasic nature of skin, is to combine two or more different membranes into a laminate or composite. Careful choice of the individual phases and the overall construction of the system should, in theory, allow the permeability characteristics to model those of real skin reasonably well.

Nacht and Yeung[43] attempted to produce a multiphasic model membrane system for skin. The system was a trilaminate consisting of alternating hydrophilic (cellulose acetate) and hydrophobic (dimethylpolysiloxane) polymeric sheets. Permeability coefficients for three permeant molecules (water, salicylic acid, and hydrocortisone) transporting across various membranes were measured and compared to those found in an identical system using human skin as the membrane. A summary of the model systems and the respective steady-state fluxes and permeability coefficients is presented in Table I. It was concluded that, for the solutes studied, none of the single-polymer systems was able to represent real skin; the best correlation was obtained with the cellulose acetate/silastic/cellulose acetate trilaminate membrane. To test further the validity of this system as a model for human skin, the permeability measurements were repeated with increasing ratios of ethanol/water as the donor solution "vehicle". Compared to permeabilities obtained when the same donor vehicles were used with excised human skin, the ratios of the membrane/skin permeability

TABLE I. Permeability Coefficients through Various Laminate Membrane Systems and through Excised Human Skin[a]

membrane	permeability coeff, cm/h		
	salicylic acid[b]	hydrocortisone[b]	water
cellulose acetate	1.83×10^{-2}	6.4×10^{-3}	
silastic surgical sheet	1.14×10^{-2}	5.3×10^{-5}	
trilaminate cellulose acetate	5.21×10^{-3}		2.6×10^{-2}
trilaminate silastic	2.72×10^{-3}		8.0×10^{-5}
multimembrane system (cellulose acetate/silastic/ cellulose acetate)	6.38×10^{-3}	2.3×10^{-5}	6.2×10^{-4}
excised human skin	6.36×10^{-3}	7.0×10^{-6}	4.2×10^{-4}

[a] Data from ref 43. [b] Donor phases were saturated solutions in 50/50 ethanol/water.

coefficients were fairly constant. For salicylic acid and hydrocortisone, these ratios were, respectively, 1.0–1.8 and 2.3–2.9. Other membrane systems evaluated in the study included single sheets of cellulose acetate annealed at various temperatures, a trilaminate of cellulose acetate alone, Diaflo ultrafiltration membranes, and both single sheets and trilaminate preparations of silastic. It was not surprising that the dialysis and cellulose acetate membranes were poor models for skin, considering that partitioning into a lipophilic phase is generally accepted as a basic mechanism for percutaneous penetration. Lamination of a porous membrane onto a partitioning phase such as silastic may better simulate the underlying aqueous dermis or viable epidermis, and their corresponding influence on solute transport, than the partitioning membrane alone. The observation that desorption of solutes out of the stratum corneum is linear with the square root of time and not with time (as a laminated structure should be)[44] is evidence that skin transport is probably dominated by diffusion through a continuous phase rather than a laminated structure and makes questionable the use of laminates as models for stratum corneum. Whether the correlation between the trilaminate membrane and excised human skin is general requires verification using a larger number of solute molecules. Unless and until this occurs, the system cannot be proposed seriously as a synthetic model for skin.

At least two different attempts to use synthetic composites as models for biological membranes have been reported. The first was a cast polymeric system containing ethylcellulose, phospholipids, cholesterol, and mineral oil. After characterization of the diffusional properties of the membrane using salicylic acid as a model penetrant,[45] the system was used to demonstrate a correlation between the in vitro rate transfer constants for a series of benzoic acid analogues and their in vivo gastrointestinal and buccal absorption.[45] Similar correlations were also shown using the solutes' water solubilities and oil/water partition coefficients.

Hunke and Matheson synthesized a series of hydrophobic (polyurethane) polymers containing blocks of hydrophilic poly(ethylene glycol) (600, 1000, or 1540).[46] The membranes allowed transport of the permeants studied (paroxypropione, 5-nitrosalicylic acid, sulfaguanidine, and phenylbutazone) at rates comparable to those of dialysis membranes, but with the selectivity of partitioning type membranes. The membrane/buffer partition coefficients were found to be much greater than one, but the high values obtained for the permeability coefficients led to the conclusion that the overall rates were determined mainly by diffusion through aqueous pores. Although no correlations to skin permeability were attempted in either of these synthetic composites, the studies do serve to illustrate the potential for "rationally designed" membrane models.

D. Zeolites

A novel approach to investigate diffusion through skin has been suggested by Dyer and co-workers.[47] The membrane consists of synthetic zeolites incorporated into a polystyrene matrix. Zeolites are aluminosilicates with a rigid three-dimensional structure. They contain about 50% of their volume as water-filled channels and cavities, and their structures have been extensively characterized. The rationale for using zeolites to model skin relies on this degree of structural characterization and on the possible similarity between solute interaction with a charged zeolite–water matrix and the charged keratin–water matrix present inside the cells of the stratum corneum. In a series of studies monitoring the self-diffusion of salicylic acid, Dyer et al. first examined the temperature dependence of diffusion through either skin (human or pig epidermis) or the synthetic membrane. The diffusion coefficient obtained through the zeolite was 1000 times greater than that found for human epidermis, and it was deduced from the computed energies of activation that human epidermis presented a higher barrier to penetration of salicylic acid than the zeolite. Further studies included a second zeolite membrane (zeolite X, which is more highly charged than the first zeolite (2.62 Y)), as well as full-thickness skin and separated dermis from both pigs and humans.[48] The calculated self-diffusion coefficients and activation parameters seemed to indicate that diffusion through the synthetic zeolite was comparable to diffusion through dermal sections rather than diffusion through either full-thickness skin or epidermis. The results confirmed that transport across the epidermis was indeed the rate-limiting process for penetration of salicylic acid through the whole skin membrane. Although synthetic zeolites certainly constitute a unique model for biological membranes, it seems unlikely that they will find practical applications in skin transport studies.

E. Silastic

The diffusional barrier of dimethylpolysiloxane (silicone rubber or silastic) was first characterized by Garrett and Chemburkar.[18] It was shown to be a nonporous partitioning membrane with a higher permeability to the molecular, rather than the ionic, species of 4-aminopropiophenone. Silastic was also shown to be impermeable to the phosphate salts contained in the buffer and to HCl. The exponential dependence of the permeability coefficient on increasing alkyl chain length for the series of alkyl *p*-aminobenzoates[49] and the series of straight-chain alkanols[50] further characterized silicone as a homogeneous hydrophobic barrier.

Poulsen and Flynn caution that the only reliable membrane for the study of percutaneous absorption is a suitable skin preparation.[40] They add that exceptions to this include studies where the purpose of the artificial membrane is to contrast specific behavior of the skin

to that of a prototype hydrophobic or watery barrier or studies that are intended to build up the basic concepts governing all permeation. With this in mind, Flynn and co-workers have included silicone rubber membranes as "perfect lipid membranes" that can be used to "control" for properties of the skin permeation not attributable to simple partitioning and diffusion.

In attempting to assess the magnitude of aqueous boundary layers and their influence on skin transport resistance, Durrheim et al. compared 1-alkanol permeabilities across silicone membranes with those through excised hairless mouse skin as a function of alkyl chain length.[50] The required property of the synthetic membrane was absence of any internal aqueous resistance (such as the viable epidermis and dermis) so that the extent of the "real" hydrodynamic boundary layer resistance could be evaluated separately and subtracted from these other "aqueous" resistances inherent in the skin. On the basis of the observed plateau in permeation through silastic for hexanol and higher homologues (indicating the onset of aqueous boundary layer control), the boundary layer thickness was calculated to be 1200 μm. The fact that this value was much greater than the 300 μm anticipated led the authors to reexamine their experimental methods. In doing so, it was discovered that as the alkanol solubility in the membrane increased, a significant portion of the applied dose was "retained" in the membrane and prohibited attainment of the pseudo-steady-state condition necessary for calculating meaningful permeability coefficients. This problem was circumvented by replenishing both the donor and receptor phase with fresh solutions at regular time intervals so that the concentration gradient across the membrane was not allowed to decay significantly. A single permeability coefficient (for octanol) was measured in this way and it appeared that permeability, under these conditions, continued to increase linearly with partition coefficient (i.e., was not yet under aqueous boundary layer control). Consequently, the boundary layer thickness was assumed to be less than 300 μm (the maximum value calculated from the "true" octanol permeability coefficient) and this upper limit was then used as the aqueous stagnant layer thickness in all subsequent calculations. By subtracting the contribution of the stagnant layers from the resistance measured through isolated dermal sections, the diffusion coefficient through this aqueous component of the skin was estimated to be about three times smaller than that through pure water. Figure 7 plots measured resistance versus the octanol/water partition coefficient for the alkanols. The general shape of the curves is similar, and it is clear from the plateau in transport resistance at high partition coefficient that membrane retention of these highly lipophilic solutes is a concern for the skin preparation as well as for the silastic membrane. It should be pointed out that, even for the most permeable solutes, the aqueous stagnant layer contribution to the overall resistance was less than 5%.

In experiments designed to assess how the skin permeability of phenol was altered by thermal injury, Behl and Flynn (again using hairless mouse skin) included silastic membranes to control for a different effect.[51] Phenol diffuses through the skin readily, and cases of systemic poisoning have been reported following topical exposure. It is known that the absorption of phenol through skin is dependent on the concentration of phenol and it has been speculated that this concentration dependence is due to chemical alteration of the skin by phenol. An alternate explanation involves a physicochemical effect in the donor solution such as complexation. Complexation would result in a more lipophilic and hence more readily absorbed species of penetrant. Silicone rubber was assumed to be chemically inert to phenol and thus was used to control for damage caused to the skin with increasing phenol concentration. Indeed, while concentrations of phenol above 2% increased the permeability of the skin both to itself and to methanol copermeant, no such effect was observed when silicone rubber was used as the barrier. It was therefore concluded that solution phenomena could not provide a mechanism for the enhanced phenol permeability found with increased concentrations of phenol. Cooper has used silicone rubber membranes to make similar conclusions about the skin penetration promoting effects of surfactants and polar solvents.[52,53]

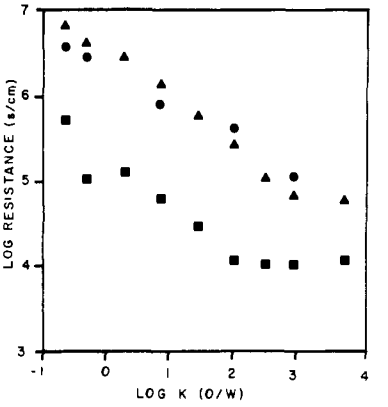

Figure 7. Resistance to solute transport for the C_1–C_{10} 1-alkanols for (▲) human epidermis, (●) hairless mouse skin, and (■) silicone rubber membranes.

F. Organic Liquid Membranes

At the present time, no synthetic membrane model for skin has been characterized and compared extensively enough to real skin to warrant an absolute claim of relevance to percutaneous absorption in vivo. A major reason for this is a lack of knowledge about the skin penetration process per se. As additional information concerning the precise macro- and microarrangement of skin components becomes available, one anticipates that synthetic membranes (perhaps in conjunction with mathematical simulations that account for alternate pathways, specific binding, and metabolism) will be designed and tested. Other problems in the field include nonstandardized conditions, under which the experiments have been conducted (i.e., compounds, dose, nature of donor and receptor phase), and the lack of perseverance on the part of many investigators to measure systematically penetration of the large number of compounds necessary to make correlations between the proposed model and human skin.

Simple organic liquids have been suggested as models for biological membranes since the turn of the century.[54,55] The rationale was based upon the observation that (as in skin penetration) the biological activity of a series of compounds increased proportionally with increases in the relative lipid/water solubility. Exam-

ples include the induction of narcosis in tadpoles,[54] the potency of anesthetic gases,[55] and the cytotoxicity of various antimicrobial agents.[56] Later, as the phospholipid bilayer/protein "fluid mosaic" arrangement of cell membranes became accepted, these observations could be defended on a physical basis. An appropriate organic phase should mimic the overall "solvent" behavior of the biological system in question. Although it is unlikely that any single organic phase would be capable of representing a complex membrane in all respects, significant correlations with biological activity have been established for a variety of simple lipids, including olive oil (esters), mineral oil (paraffins), and pure organic solvents (such as alkanes, ether, and chloroform).[56,57] Hansch and others have suggested 1-octanol as a rational model solvent.[56] This suggestion reflects the possible coincidence between molecular interactions of a solute molecule and the biological membrane (polar protein and phospholipid headgroup components, nonpolar lipid hydrocarbon interior) and those of the solute and octanol (polar hydroxyl group, nonpolar C_8 hydrocarbon). More work has been performed with octanol as the model solvent than any other, and the number of compounds with known octanol/water partition coefficients is extensive.[58] The octanol/water partition coefficient thus provides an easily obtained (often directly from the literature) measure of relative solute lipophilicity and is widely used as such in quantitative structure–activity analysis.

In skin penetration work, although octanol has been used as a "lipophilicity index" for some structure–penetration correlations, the solvent has not been adopted as a membrane model per se for transport studies. More appropriately (and, often, fortuitously), organic liquids or other materials of greater hydrophobicity than octanol have been employed. We now review the chronological development of this avenue of research.

1. Early Work

Preliminary studies involved evaluation of the kinetics of drug transfer through bulk organic phases.[17,59] These investigations were designed to simulate gastrointestinal absorption of drugs and to establish the importance of partitioning, pH (for ionizable drugs), drug structure modification, the specific organic phase employed, viscosity of the organic phase, and the solubility parameter of the organic phase relative to the solute.

Poulsen[60] suggested that IPM was more representative of skin lipids than the rather hydrophilic liquids that had been used previously (e.g., glycols), and a reasonable amount of work has been pursued under this assumption. The simplest experiments have used IPM as a bulk receptor phase to measure drug release from various topical formulations.[61] Incorporation of the organic phase into the pores of a filter paper creates a membrane model that can be used in solute transport studies.[62] Tanaka et al.[63] have used a Millipore filter filled with the organic liquid to measure the permeation of salicylic acid and aminopyrene through different lipid phases. The pH dependence of the process was confirmed for the two drugs using olive oil in the membrane. The dependence of the diffusion coefficient of salicylic acid in olive oil on temperature and the effects of changing the organic phase employed (olive oil, liquid paraffin, cyclohexane, and oleic acid) on the observed drug permeability were also investigated.

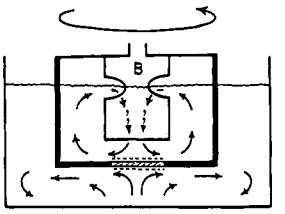

Figure 8. Cross-sectional diagram of the RDC. The model skin membrane (hatched region) consists of a Millipore filter impregnated with the organic phase. Initially, the inner compartment contains an aqueous solution of penetrant and the outer compartment consists of distilled water (or buffer). Solute flux is measured by spectrophotometric analysis of the receptor phase as a function of time. Rotation of the inner (donor) compartment establishes spinning-disk hydrodynamics (see flow lines) in the outer (receptor) compartment. The stationary baffle (B) in the inner compartment ensures that identical hydrodynamics are also imposed on the aqueous donor solution. The thickness of the aqueous stagnant layers (dashed lines) on either side of the rotating filter are calculable from the cell rotation speed; hence their contribution to the total transport resistance can be determined and subtracted.

2. Rotating Diffusion Cell (RDC)

The RDC has been employed most frequently in model skin penetration studies using organic liquids. The RDC is a relatively new method for characterizing transport phenomena[64] and offers certain advantages over other more conventional diffusion cells. These attributes include the versatility offered by a model organic phase which is easily changed and the design of the system which allows the individual contribution of each of the various physical steps involved in the transport to be evaluated.

A diagram and description of the RDC are given in Figure 8. A Millipore filter divides the inner (donor) and outer (receptor) compartments of the cell and is impregnated with the model organic phase. Rotation of the inner compartment establishes spinning-disk hydrodynamics in the receptor and donor compartments and creates stagnant diffusion layers of known thickness in the aqueous phases adjacent to the filter. The flow lines describing the established hydrodynamics are included in Figure 8. The thickness of the aqueous stagnant layers is a function of rotation speed and is given by[65]

$$Z_D = 0.643\nu^{1/6}D_{aq}^{1/3}\omega^{-1/2} \quad (1)$$

where ν is the kinematic viscosity of the aqueous phase, D_{aq} is the aqueous diffusion coefficient of the solute, and ω is the rotation speed of the filter. The concentration profile for a solute transporting across the RDC filter system is diagrammed in Figure 9. The equation in the diagram is obtained by solving the steady-state flux equations for each step in the transport process. Each term in the expression corresponds to the physical step shown directly above that term in the diagram.

Solute flux across the model membrane is measured at different rotation speeds, and the resulting solute transport resistance is plotted against the inverse square root of rotation speed. The intercept of this plot corresponds to infinite rotation speed and, hence, to zero aqueous stagnant layer thickness. The total resistance here is then comprised only of the resistances to transfer

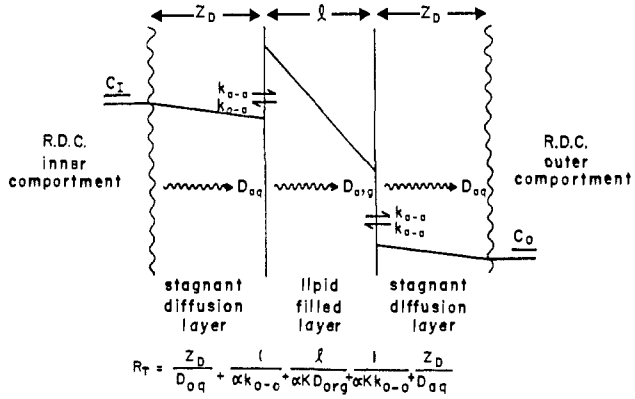

Figure 9. Concentration profile for a solute transporting across the RDC filter system. Each physical step in the overall transfer of a solute molecule from bulk donor to bulk receptor solution presents a resistance to transport, and the total resistance is the sum of these individual components. The five steps are (1) diffusion through the donor compartment aqueous stagnant layer, (2) partitioning into the lipid phase within the filter, (3) diffusion across the model membrane phase in the filter, (4) partitioning out of the organic phase into the aqueous receptor solution, and (5) diffusion through the receptor compartment aqueous stagnant layer. The transport process is described mathematically by solving the appropriate steady-state flux equations for the individual steps. The resulting expression for the total transport resistance in terms of component resistances is given below the concentration profile. Each term in the equation corresponds to the physical step below which it appears.

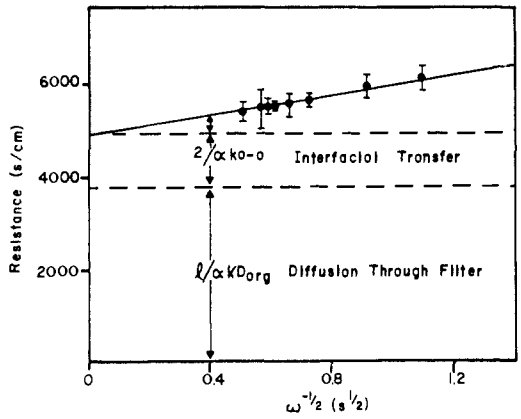

Figure 10. Total resistance to solute transport (R_T) in the RDC as a function of rotation speed (ω). Equation 1 (see text) predicts a linear dependence of R_T on $\omega^{-1/2}$. Extrapolation of the total resistances obtained at different rotation speeds to $\omega^{-1/2} = 0$ (i.e., infinite rotation speed) eliminates the aqueous stagnant layer resistance and yields an intercept that describes the sum of membrane diffusion and interfacial transfer resistances. The magnitude of each of these components for methyl nicotinate transporting across an IPM-impregnated filter is indicated on the graph. The extrapolated line has the theoretical slope predicted by eq 1, and its coincidence with the experimental data confirms that the correct hydrodynamics have indeed been established in the RDC.

into and out of the membrane ($2/k_{ao}\alpha$) and to diffusion through the organic phase ($h/D_oK\alpha$); k_{oa} is the aqueous → organic interfacial transfer rate constant of the solute, K is the solute's organic/aqueous partition coefficient, D_o is the diffusion coefficient of the solute in the organic phase, and h and α are, respectively, the thickness and porosity of the filter. Figure 10 shows a typical "Levich plot" and indicates the contribution provided by each of the individual resistances.

Albery and Hadgraft[66] first used the RDC to estimate the possible contribution of interfacial transfer barriers to epidermal penetration. With a knowledge of the magnitude of these barriers, it is possible to estimate whether they are capable of limiting the rate of the overall process. IPM was used as the model organic phase for skin lipids, and transport of the methyl, *n*-butyl, and *n*-hexyl esters of nicotinic acid, plus acetic acid and *p*-methylbenzyl chloride, was studied. Substantial free energy barriers for the transfer reaction were found even when the overall process was thermodynamically favorable. However, it was concluded, from this and extensive in vivo work, that, if the size of the phase-transfer rate constants in percutaneous penetration were similar to those measured in this model system, then interfacial transport was unlikely to be the rate-determining process. It was suggested, though, that the observed free energy barriers would be sufficiently large to control, at least in part, transport across biological membranes of smaller width than the stratum corneum.

TABLE II. Diffusion Coefficients Estimated with the RDC for Methyl and Ethyl Nicotinate in Various Organic Phases[a] and in Human Stratum Corneum[b]

	est $D_{org}/10^7$, cm^2 s^{-1}	
organic phase	methyl nicotinate	ethyl nicotinate
IPM	51.0	46.0
tetradecane	110.2	81.7
linoleic acid	14.3	12.0
2% egg lecithin in IPM	20.3	15.9
1% DL-β,γ-dipalmitoyl α-lecithin in IPM	16.1	14.0
stratum corneum[b]	2	

[a] Data from ref 68. [b] From in vivo data in ref 69.

Guy and Fleming have used the RDC to estimate the diffusion coefficients of methyl and ethyl nicotinate through a series of organic barriers.[67] The percutaneous absorption of the solutes had previously been studied in vivo by observing the time taken for erythema (redness caused by local dilatation of blood vessels) to develop after their application to the skin.[68] Mathematical analysis of the data allowed stratum corneum diffusion coefficients to be estimated, and these were used as "control" values to assess the possible utility of the organic phases tested as model skin membranes. The organic barriers included IPM, tetradecane, linoleic acid, 2% egg lecithin in IPM, and 1% dipalmitoylphosphatidylcholine in IPM. The calculated diffusion coefficients (D) through each of the model barriers and through stratum corneum are shown in Table II. All calculated diffusion coefficients were greater than the 2×10^{-7} cm^2 s^{-1} value previously estimated for diffusion through stratum corneum and suggested that the systems with the smallest calculated value of D (i.e., linoleic acid or dilute solutions of phospholipid in IPM) were better models for studying diffusion of small solutes across skin. To calculate the diffusion coefficients it was assumed that the rate constant for transfer from the aqueous donor solution into the membrane phase was independent of the organic liquid. The assumption was defended by using the argument that all the organic phases had a large hydrocarbon component and that this would primarily dictate the solute transfer rate. No evidence to support the hypothesis was presented and its validity must be questioned, therefore, particularly when surface-active compounds are incorporated into the lipid phase. If the relative resistance to solute

penetration for the above systems is considered, rather than the diffusion coefficient within the organic phase, it may be shown that tetradecane presents the largest barrier (rather than the weakest barrier suggested by the large estimated diffusion coefficient) and one might conclude, as a result, that tetradecane models the skin better than the other four more permeable lipid phases (see "3. Latest Studies," below). From a practical standpoint, it is probably more sensible to compare the overall barrier properties of the membrane model to those of skin since, in percutaneous absorption, it is frequently the total permeation that one wishes to predict.

In a subsequent set of experiments, the RDC membrane was impregnated with crystalline phospholipid.[69] The phospholipid was deposited in the filter by repeatedly applying a dilute chloroform solution of dipalmitoylphosphatidylcholine (allowing the solvent to evaporate between applications). The solutes were again methyl and ethyl nicotinates. In this case, rather than calculating and comparing diffusion coefficients through the model barrier to those for stratum corneum, effective permeability coefficients (P) were compared. For methyl and ethyl nicotinates crossing the lipid membrane, the P values were 1.1×10^{-4} and 2.8×10^{-4} cm s^{-1}, respectively, and compared favorably with the 0.5×10^{-4} cm s^{-1} value determined in vivo for the methyl ester crossing the stratum corneum. It was pointed out that the relative contributions to the overall permeability coefficient (i.e., resistance due to interfacial transfer into and out of the membrane versus diffusion through the barrier) for the lipid barrier do not necessarily parallel those for the skin and that further work with different penetrants must be performed to assess whether the correlation is a general one.

Hadgraft et al. have used the RDC to investigate the ability of the skin penetration enhancer 1-dodecylazacycloheptan-2-one (Azone) to facilitate the transfer of sodium salicylate across an IPM-impregnated filter.[70] Azone has been shown to promote the percutaneous penetration of various hydrophobic and hydrophilic chemicals.[71] The exact mechanism by which Azone accomplishes this feat is not well understood but has been thought to involve either increasing the thermodynamic activity of the drug in the vehicle (so as to promote transfer of the drug into the stratum corneum) or altering directly the barrier properties of the stratum corneum.[27] Azone, it should be noted, is an effective penetration enhancer even when present in concentrations as low as 1%. Because the ring nitrogen of Azone can be protonated, Hadgraft and colleagues suggested that protonation followed by ion pairing with negatively charged salicylate ions could result in facilitated transport of the pair. The surface of the skin is slightly acidic (pH 4.2–5.6), whereas the lower layers of the epidermis are maintained at physiological pH (7.4).[72] Hence, in the study described, such a pH gradient across the model membrane was used to drive the transport process in the following putative fashion: At the lower pH of the donor compartment (5.0) the carrier is protonated and can combine with the anions present to form ion pairs in the interfacial region. The ion pair then partitions into the bulk lipid phase and diffuses down its concentration gradient to the opposite interface. At the higher pH of the receptor solution (7.4) the carrier deprotonates and releases the anion. It was found that salicylate transport was increased by a factor of 1.7 over IPM alone when 0.1 M Azone was incorporated into the organic barrier; it was suggested, therefore, that ion pairing of anionic drugs may be an important mechanism for the observed penetration enhancement induced by Azone incorporated into topical drug delivery systems. In addition to Azone, Barker and Hadgraft[73] have demonstrated the ability of various other carrier amines, including *N*,*N*-bis(2-hydroxypropyl)octadecylamine, *N*,*N*-bis(2-hydroxybutyl)hexadecylamine, *N*,*N*-bis(2-hydroxybutyl)octadecylamine, *N*-(2-hydroxypropyl)bis(octadecyl)amine, and Ethomeen S12 to facilitate the transport of anionic molecules (salicylic acid, resorcin brown R, and methyl orange) when incorporated into the IPM phase of the RDC. The degree of facilitation was shown to depend both on the carrier amine and on the particular solute being transported. In all cases, the transport was found to be saturable, as would be expected of a carrier-mediated process. Ethomeen S12 was able to increase the transport rate of salicylic acid through IPM by an order of magnitude. It was also noted, in preliminary work using a rabbit model for percutaneous penetration, that the penetration of salicylate ions could be enhanced by at least an order of magnitude using these same carrier amines.

3. Latest Studies

Because of increasing evidence that diffusion through the continuous intercellular lipid matrix of the stratum corneum is the major transport route through this tissue, it is reasonable to suggest that a membrane containing these lipids would be a relevant model for permeation studies. Firestone and Guy[74] initiated such an approach using the RDC with a lipid mixture containing phosphatidylethanolamine (3%), dipalmitoylphosphatidylcholine (7%), ceramides from bovine brain cerebrosides (6%), ceramides from bovine brain sphingomyelin (6%), cholesterol (25%), cholesterol palmitate (26%), linoleic acid (9%), and tristearin, (17%). (Compare this to the known composition of stratum corneum intracellular lipid given in Figure 2.) As with previous work using crystalline lipids, a dilute (2%) solution of the lipid mixture in chloroform was applied to the filter and the solvent allowed to evaporate. This was repeated until the filter was completely saturated with lipid. This mixture is an oily white solid at 25 °C (the temperature at which the experiments were carried out) but the precise arrangement of the lipids within the filter has not been characterized. It is not known whether the lipids in membranes prepared this way would assume a bilayer configuration and the ultimate arrangement will most certainly depend on the amount of water present in the membrane. Furthermore, it remains to be seen whether the bulk lipid properties or the detailed molecular arrangement of the stratum corneum intercellular lipids is the more important determinant of solute permeation through this phase.[27]

For a limited number of compounds (methyl nicotinate, ethyl nicotinate, phenol, *p*-methoxyphenol, *p*-nitrophenol, and 4-chloro-*m*-cresol), an apparently linear relationship (slope = −0.31; 95% confidence limits = ±−0.23) between the model membrane resistance and

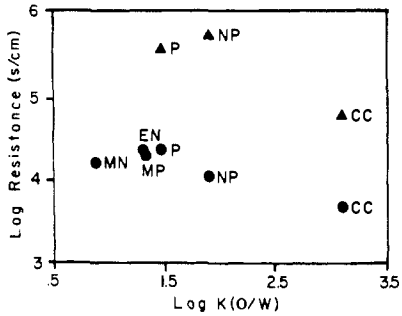

Figure 11. Relationship between transport resistance across a model skin lipid membrane (●) and solute octanol/water partition coefficient.[74] The organic phase was a mixture of lipids mimicking those actually present in the intercellular domain of stratum corneum.[1] The solutes studied were methyl nicotinate (MN), ethyl nicotinate (EN), *p*-methoxyphenol (MP), phenol (P), *p*-nitrophenol (NP), and 4-chloro-*m*-cresol (CC). For three solutes (P, NP, and CC), the corresponding transport resistances across human epidermis in vitro (▲) are also shown.[14]

TABLE III. Solutes Studied in Recent RDC Model Skin Membrane Systems and Their Corresponding Organic Phase/Aqueous Partition Coefficients[a]

compd	log $K_{o/w}$	log $K_{I/w}$	log $K_{TD/w}$
resorcinol	0.80	-0.32	
catechol	0.88	0.10	-2.73
p-methoxyphenol	1.37	0.88	-1.17
phenol	1.46	1.06	-0.95
p-cresol	1.94	1.55	-0.41
p-bromophenol	2.59	2.36	-0.17
p-iodophenol	2.91	2.40	0.07
4-chloro-*m*-cresol	3.10	2.57	0.30
n-butylphenol		3.2	1.32
n-pentylphenol		3.7	1.87
methyl nicotinate	0.83	0.28	-0.21
ethyl nicotinate	1.32	0.89	0.37
n-butyl nicotinate	2.04	2.04	1.61
n-pentyl nicotinate	2.55	2.34	2.01
n-hexyl nicotinate	3.51	2.96	2.76

[a] $K_{o/w}$ = octanol/water partition coefficient; $K_{I/w}$ = IPM/water partition coefficient; $K_{TD/w}$ = tetradecane/water partition coefficient.

the substrate octanol/water partition coefficient ($K_{o/w}$) was found (Figure 11). The fluxes of three more hydrophilic solutes (1,3-dihydroxybenzene, nicotinic acid, and nicotinamide) were so slow as to be nondetectable by UV analysis of the receptor phase over a reasonable period of time. The shallow slope of the data in Figure 11 indicated that flux through the model system was relatively insensitive to the degree of lipophilicity of the penetrant over the range of $K_{o/w}$ considered (1.0–3.1). When the solute was more hydrophilic ($K_{o/w} < 1$), transport resistance increased dramatically and could not be characterized by the methods used in the study. These considerations made the value of further work with the complex mixture of lipids questionable until both the system and the lipid mixture were better characterized.

The problems associated with the above system together with a need (a) to establish a simple model screen for percutaneous absorption and (b) to conduct an initial, well-controlled, study of structure–permeability relationship in general, led Houk and Guy[20] to consider the transport of two series of structurally related compounds through two simple lipid barriers.

The solutes studied in our recent work comprise five esters of nicotinic acid and ten phenol derivatives. The

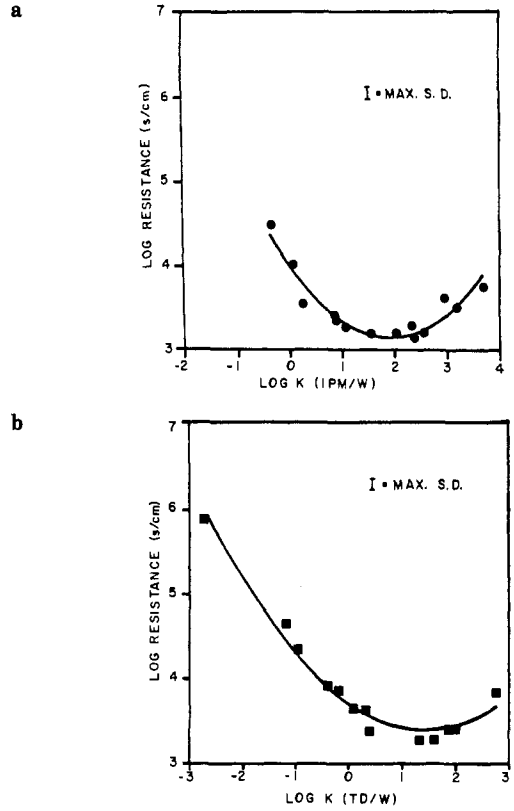

Figure 12. Transport resistance across RDC model skin membranes versus solute organic liquid/water partition coefficient.[20] The model organic liquid membranes were (a) IPM and (b) TD. Each point represents a mean resistance (±standard deviation) determined from approximately 25 separate flux measurements. The smooth curves on the graphs are the least-squares fit of the data assuming a quadratic relationship between resistance and partition coefficient. The equations describing the curves are as follows. IPM: $\log R_T = 0.24(\pm0.02)(\log K)^2 - 0.91(\pm0.08)(\log K) + 4.04(\pm0.06)$; $r^2 = 0.912$. TD: $\log R_T = 0.15(\pm0.01)(\log K)^2 - 0.43(\pm0.02)(\log K) + 3.72(\pm0.04)$; $r^2 = 0.972$. (The numbers in parentheses are the standard deviations for the corresponding coefficients.) The solutes and corresponding partition coefficients are listed in Table III.

molecules are specifically identified in Table III, which also includes, for each compound, its octanol–water, IPM–water, and tetradecane (TD)–water partition coefficients. These values either were measured experimentally in the conventional way[75] or were obtained from the literature.[58]

In Figure 12, the experimental transport resistances for the 15 solutes across IPM and TD barriers in the rotating diffusion cell at 25 °C are plotted against the respective organic liquid–aqueous phase partition coefficients. Each point on the curves represents a mean resistance (±standard deviation) determined from approximately 25 separate flux measurements. The smooth curves on the graphs represent a computer fit to the data assuming an empirical quadratic relationship between the logarithm of the transport resistance (R_T) and the logarithm of the corresponding partition coefficient (K):

$$\log R_T = \alpha + \beta(\log K) + \gamma(\log K)^2 \qquad (2)$$

The coefficients, α, β, and γ, are given in the legend to Figure 12.

The functional dependence of $\log R_T$ on $\log K$ is very consistent between the two skin lipid models. It appears that TD presents an overall greater resistance to

TABLE IV. Octanol/Water Partition Coefficients ($K_{o/w}$) and Transport Resistances through Excised Human Skin in Vitro for 19 Phenol Derivatives[a]

solute	log $K_{o/w}$	skin transport resistance/10^4, s/cm
resorcinol	0.80	1500
phenol	1.46	43.8
4-nitrophenol	1.96	64.5
3-nitrophenol	2.00	63.8
p-cresol	1.95	20.5
o-cresol	1.95	22.9
m-cresol	1.96	23.6
methyl hydroxybenzoate	1.96	39.5
2-chlorophenol	2.15	10.9
3,4-xylenol	2.35	10.0
4-chlorophenol	2.39	9.91
4-ethylphenol	2.40	10.3
4-bromophenol	2.59	10.0
β-naphthol	2.84	12.9
2,4-dichlorophenol	3.01	5.99
chlorocresol	3.10	6.55
thymol	3.34	6.82
chloroxylenol	3.39	6.10
2,4,6-trichlorophenol	3.69	6.06

[a] Data from ref 12.

transport than IPM for all penetrants considered, the difference being greatest for the more hydrophilic examples. The log R_T versus log K relationships are markedly nonlinear. The fact that R_T does not continue to decrease monotonically with increasing K suggests that simple membrane diffusion does not control the overall transport rate for the most lipophilic compounds. The design of the rotating diffusion cell apparatus precludes the classic interpretation that the stagnant aqueous diffusion layer resistance (R_{SL}) is rate limiting at high log K. R_{SL} can be determined specifically and can be shown to be insignificant under the conditions of this experiment. The remaining potential contribution to R_T is the resistance to solute transport across the aqueous phase–organic liquid interfaces. Indeed, the rotating diffusion cell was originally developed[64] to measure the kinetics of this phase-transfer process; it has since been used to demonstrate the significance of the transport step in membrane permeation.[20,66] The parabolic form of the log R_T versus log K curves suggests to us that slow partitioning out of the lipid membrane has become the rate-limiting step for the most lipophilic permeants. However, although the transport data imply apparent steady-state behavior (i.e., the number of moles arriving in the outer compartment per unit time is constant), it is probable that a true steady state does not exist. For very lipophilic molecules, there is rapid and substantially complete uptake from the aqueous inner compartment into the lipid membrane. Experimentally, then, we measure the slow, essentially zero-order, release of the permeant from the lipid "slab". When the kinetics of partitioning are very slow (the case for highly lipophilic molecules), the rate of this release process is constant.[76]

The pattern of behavior seen with the phenols may be compared to published data[15] on the transport resistance of excised human cadaver skin to the derivatives listed in Table IV. Although the analogues studied do not overlap perfectly with those considered in our RDC work, the similarity between the form of the model membrane data and that of the in vitro skin resistances is striking (Figure 13). In this plot, we have used the octanol–water partition coefficient ($K_{o/w}$) as

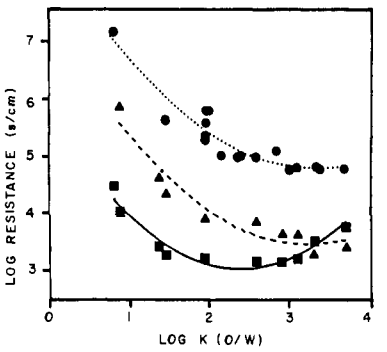

Figure 13. Comparison of solute transport resistance across model skin lipid membranes[20] with those obtained across excised human skin in vitro[14] for a series of phenol derivates. The observed resistances are plotted against the solute octanol/water partition coefficient as a common measure of lipophilicity. The model barriers were IPM (■) and TD (▲), and the solutes were the series of phenols listed in Table III. For excised human skin (●), the solutes and their corresponding resistances and partition coefficients are listed in Table IV. The smooth curves represent least-squares fits of the results assuming a quadratic relationship between resistance and partition coefficient. The coefficients α, β, and γ of the fits (see eq 2) are given in Table V.

TABLE V. Coefficients[a] α, β, and γ (eq 2) Describing the Quadratic Relationships between log R_T and log $K_{o/w}$ Shown in Figure 13

membrane	α	β	γ	r^{2b}
RDC–IPM[c]	5.80(±0.26)	−2.32(±0.28)	0.48(±0.06)	0.917
RDC–TD[c]	7.53(±0.61)	−2.55(±0.59)	0.40(±0.13)	0.923
excised human skin[d]	8.74(±0.40)	−2.39(±0.35)	0.36(±0.07)	0.889

[a] Values were found by least-squares analysis and are presented with their standard deviation. [b] r = correlation coefficient. [c] Data from ref 20. [d] Data from ref 14.

a common measure of solute lipophilicity. Again, the smooth curves represent least-squares fits of the results assuming a quadratic dependence of log R_T on log $K_{o/w}$. The coefficients α, β, and γ (see eq 2) are given in Table V. While it is clear that the model membranes underestimate actual skin resistance, they do provide quite useful relative indications of permeability. An intriguing difference between the model systems and the skin membrane is suggested by the low log $K_{o/w}$ region and is reflected in the intercept term (α) which corresponds to the transport resistance of a phenol with log $K_{o/w} = 0$. The α parameters imply that skin becomes relatively more forgiving than the pure organic liquids to the passage of more water-soluble compounds. It appears that, while skin offers a greater resistance to transport than IPM and TD for phenols in the log $K_{o/w}$ range 1.0–3.5, the relative ordering is reversed as log $K_{o/w}$ decreases below 1.0. This inference is consistent with the proposed existence of parallel polar and nonpolar pathways across stratum corneum. As stated previously, although the structural origin of these routes has not been established, there is physicochemical evidence to support the hypothesis.[9,11] Obviously, simple organic liquids cannot provide a continuous "polar" pathway through the 150-μm width of the filter support in the RDC. If skin contains a low-capacity hydrophilic pathway, discrepancy between the models and the biological tissue would be expected for more polar penetrants. The mechanisms of percutaneous transport remains an avidly studied field at this time and further endeavor is warranted.

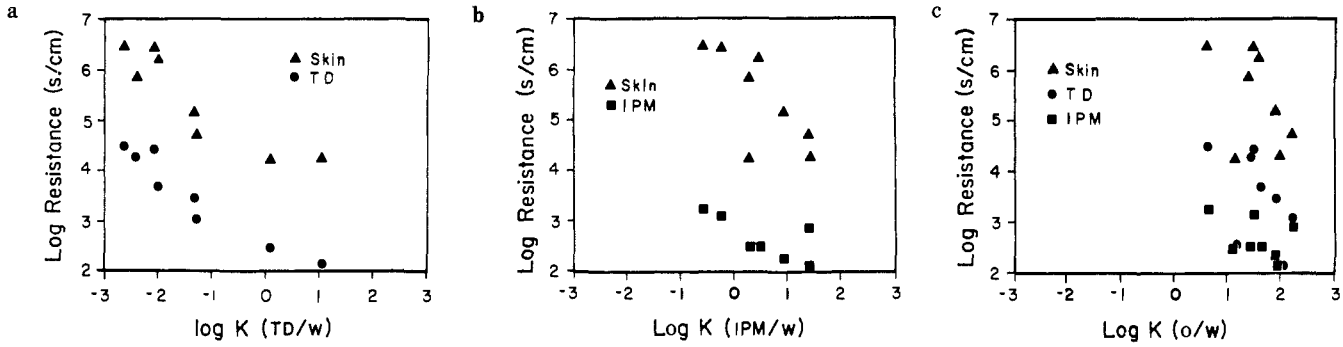

Figure 14. Transport of an unrelated series of compounds ((1) barbital, (2) phenobarbital, (3) butabarbital, (4) amobarbital, (5) hydrocortisone, (6) salicylic acid, (7) nicotine, and (8) isoquinoline) across IPM and TD model membranes in the RDC, and excised human epidermis in vitro.[77,78] (a) TD resistances compared to epidermal resistances as a function of solute TD/water partition coefficient; (b) IPM resistances compared to epidermal resistances as a function of solute IPM/water partition coefficient; (c) TD and IPM resistances compared to epidermal resistances as a function of solute octanol/water partition coefficient.

In experiments related to our work with phenols and nicotinates, Ridout and Hadgraft[77,78] have compared transport of a group of unrelated compounds across TD and IPM in the rotating diffusion cell with their penetration through excised human epidermis in vitro. The molecules studied were barbital, phenobarbital, butabarbital, amobarbital, hydrocortisone, salicylic acid, nicotine, and isoquinoline. In Figure 14, the in vitro skin resistances are compared to the corresponding transport resistances across tetradecane and isopropyl myristate model membranes, respectively. In Figure 14a, the ordinate is the solute TD/water partition coefficient ($K_{TD/w}$). In Figure 14b, it is the IPM/water concentration ratio at equilibrium ($K_{I/w}$). For the TD barrier, R_T is observed to decrease with increasing $K_{TD/w}$. The parabolic form of the profile seen in our studies (Figure 12) is not apparent here, though it should be emphasized that the most lipophilic substance had a log $K_{TD/w}$ of only slightly greater than 1.0. The minimum in our curve for phenols and nicotinates crossing TD was at log $K_{TD/w}$ = 1.4. The shapes of the curves, however, again support the usefulness of the model for estimating relative penetrabilities. The comparison with IPM supports similar basic observations; again, IPM, on the whole, offers less resistance to solute transport than TD. The solutes studied span a much narrower range of log $K_{I/w}$ than log $K_{TD/w}$ and the results are compressed into 2 log units of $K_{I/w}$ (compared to nearly 4 for $K_{TD/w}$). This observation reflects the increased potential for the solute to hydrogen bond in IPM as compared to TD. For more polar solutes, therefore, IPM is capable of taking up more molecules per unit volume than TD and the partition coefficient is correspondingly greater. The effect is reinforced in Figure 14c, in which skin, TD, and IPM resistances are plotted against solute octanol/water partition coefficient $K_{o/w}$. The range of $K_{o/w}$ now covers a much attenuated span and the effect of obvious trends is difficult to discern. It would appear, therefore, that the success of a model to predict solute penetrability as a function of lipophilicity may be dependent upon the index (e.g., partition coefficient) chosen to represent the latter.

On the whole, progress in the use of simple organic liquid membranes as models for skin penetration studies has continued. Sufficient data have now been generated in the model systems and across real skin in vitro to allow comparisons which enable some validation to be performed. The simplicity of the system is attractive and its ability to mimic slow stratum corneum to viable epidermis transport for highly lipophilic moieties is realistic and of much value. Limitations exist, of course, not the least of which is that a simple organic liquid cannot adequately simulate complexities of skin such as parallel transport pathways. Nevertheless, as a screening mechanism for potential topical drugs or (on the other hand) likely dermal toxicants, the use of these simple systems should be further pursued.

G. Related Developments

Within the past few years, an important number of developments have taken place in the percutaneous absorption field. While these investigations have not necessarily included the identification of model membranes specifically, they have provided significant advances in our understanding of the skin's permeability barrier. Hence, this research will ultimately impact, it is believed, on the evolution of future membrane models of greater relevance and applicability.

Revelation of the nature and organization of the intercellular lipids of the stratum corneum represents a particularly crucial advance.[79] Together with the evidence described earlier, it now seems very probable that the tortuous, intercellular domain provides the dominant pathway through the skin's outermost layer. The fluidity of the intercellular lipid appears to be a marker for the permeability barrier; this conclusion is supported by the elegant work of Potts et al.,[29] who correlated molecular information (from differential scanning calorimetry and Fourier transform infrared spectroscopy) on the intercellular lipid with measurements of solute transport resistance through skin in vitro. They further showed that agents capable of enhancing percutaneous transport rates also increased intercellular lipid fluidity. With the use of attenuated total internal reflectance IR spectroscopy, experiments such as these can be conducted in vivo.[80] This increasing molecular and mechanistic understanding will certainly contribute to the selection of future membrane models.

Another intriguing development resulting from characterization of the intercellular lipids of the stratum corneum has been reported by the laboratory of Downing and Wertz.[81,82] Formation of liposomes (hollow lipid vesicles) from intercellular stratum cor-

neum lipids has been described. Although initial work has focused upon the morphological characterization of these structures, they clearly represent a model system of considerable potential for transport studies.

The ultimate model, of course, would combine the uniformity and reproducibility of an artificial system while retaining the biological significance of a recently excised piece of skin tissue. Cell culture theoretically offers this ideal combination and recent advances using keratinocytes have brought this objective much closer to realization. Proliferation of conventional primary keratinocyte cultures has not proved difficult, and a simple modification of the approach has allowed formation of a fully differentiated epidermal layer including a morphologically distinct stratum corneum.[83] To achieve this end, once the keratinocytes have achieved confluence in a conventional submerged culture, their upper surface is then exposed at the air–liquid interface. The cells are now fed only from below (mimicking the in vivo situation) and continued replication in the medium advances the upper cells out of the culture fluid. Terminal differentiation ensues within several days, leading to a structurally characteristic stratum corneum consisting of multiple cell layers. Lamellar bodies (membrane coating granules) which extrude their contents into the intercellular spaces of the lower stratum corneum in vivo are consistently identified in these systems.[84] At this time, the approach has been most successful using neonatal murine (rather than human) keratinocytes. Additionally, the morphological evidence for the existence of a representative barrier has not yet been substantiated by transport measurements. Nevertheless, there is no question that this innovation, of many possible, has the greatest potential to render all others obsolete.

An alternative option of similar attractiveness would involve the establishment of a validated in vivo animal model. The requirements here are that the animal be easy to work with, that its skin be representative of human skin, and that it facilitate meaningful assessment of variables (such as cutaneous metabolism, the role of blood flow, etc.) which are typically difficult or impossible to measure in conventional in vivo experiments. Progress toward these objectives has been documented by Krueger and co-workers.[85] Although the system developed is not simple to set up and maintain, it does provide a very relevant skin membrane (human epidermis transplanted onto an athymic nude rat) arranged such that it is isolated with an independent but experimentally accessible vasculature. Briefly, the model is formed as follows: A skin flap is raised on the abdomen of a congenitally athymic (nude) rat and a split-thickness human skin graft is sutured to the subcutaneous surface of the rat skin flap. After approximately 3 weeks, using careful microsurgical techniques, the skin "sandwich" is isolated, with its supplying vasculature, transferred to the back of the animal through a subcutaneous tunnel, and sutured in place. An "in vivo" isolated perfused organ (i.e., the skin) is thus obtained. Providing that the percutaneous absorption of test compounds of interest is relatively rapid, transport (and, in theory, skin metabolism, for example) can be determined by differential analyses of the blood supplying and draining the skin "sandwich". Blood supply to the flap can be monitored and varied with ease. Present limitations of the system include the complex surgery necessary and the requirement that the animals receive immunosuppressive chemotherapy. The approach is best suited to chemicals that are efficiently absorbed across skin; analytical sensitivity is problematic when transdermal passage is slow. Nevertheless, this technique is an important advance that offers much promise.

Finally, all model systems ultimately rely for their validation on reliable measurements of the "real" event that they are attempting to simulate. The standard approach to determine in vivo percutaneous absorption has involved an indirect radiochemical method.[86] Recently, this methodology has been improved such that complete accountability of the chemical dose is achieved and a much more reliable evaluation of skin penetration rate and extent is thereby attained.[87] Alternative procedures for monitoring in vivo percutaneous absorption have also been developed and refined. For example, two noninvasive procedures can be cited: Laser Doppler velocimetry[88] monitors changes in skin blood flow caused by transdermally absorbed vasoactive molecules.[89] If a relationship between this pharmacological effect and the concentration of the active species can be established or assumed, then skin transport kinetics can be deduced.[90] The disappearance of chemicals from the skin surface has also been shown to correlate with more classical evaluations of percutaneous absorption.[87] The judicious application of this approach, therefore, may also permit facile evaluations of in vivo penetration to be obtained.

V. Conclusions

The penetration of chemicals across the skin of man has assumed heightened significance in recent years. The successful delivery of systematically active drugs via the skin has provoked considerable activity in the pharmaceutical area. Indeed, the potential therapeutic and economic advantages perceived has led to the formation of a number of companies, the sole activity of which is transdermal drug delivery. In occupational and environmental toxicology, there is now general recognition that the skin represents an often important route of ingress of noxious materials. The evaluation of dermal exposure levels and the associated risks to the individuals involved has become a key activity for both chemical producers and government regulatory authorities.

The need is clear, therefore, for suitable model systems to study percutaneous absorption and to predict skin penetration for new materials or for existing compounds from new delivery vehicles. There is not, unfortunately, at this time, a generally accepted "best" model system. Those that have been tried have ranged from very simple to quite complex and have covered a spectrum of apparent relevancy to the tissue of interest, namely skin. The ideal system will, of course, maximize simplicity and relevancy and will have to demonstrate extensive characterization and validation. It will also need to exhibit flexibility with respect to the chemical exposure and drug delivery situations that it will be expected to simulate. The work performed so far serves to indicate those avenues of research that will prove most fruitful and useful to the ultimate objective of an acceptable model. This review has attempted to high-

light the problems that exist, to assess critically the serious attempts that have been made in the area, and to indicate the paths that will surely form the focus of new endeavors to identify representative model membranes for skin penetration studies.

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