Journal of Pharmaceutical Sciences

JUNE 1975

VOLUME 64 NUMBER 6

REVIEW ARTICLE

Percutaneous Absorption

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Keyphrases □ Percutaneous absorption—review of *in vitro* and *in vivo* penetration methods, skin transport, physicochemical factors, sorption promoters □ Absorption, percutaneous—review of *in vitro* and *in vivo* penetration methods, skin transport, physicochemical factors, sorption promoters □ Skin transport—review of percutaneous absorption

CONTENTS

The skin, the largest organ of the body, is under constant assault by numerous chemical and physical agents. New synthetic chemicals, toxic gases and liquids from chemical warfare research, environmental and industrial hazards, cosmetics, and topical medicaments penetrate the protective epidermis, producing either local or systemic toxicity.

Much work has been done to elucidate skin structure, physiology, barrier properties, and the mechanisms by which substances enter and cross the skin (1). Yet only in the last 2 or 3 decades has the scientific study of percutaneous absorption moved from emphasizing descriptive detail to correlating physicochemical factors in attempts to understand which molecules may be expected to breach the skin barrier. Studying the types of epidermis involved, the nature of the biochemical and physiological activities of the epidermis, and the epidermal barrier unit should indicate how to manipulate structure to cause selective and effective permeability.

In particular, valuable information on the influence of vehicles is just beginning to evolve. The potential exists for developing vehicles that will enable the drug to reach the site of action rapidly and maintain a sufficient concentration at the site for the required length of time. With increased understanding of percutaneous absorption, we can expect to see the advent of singularly effective new topical drugs—

drugs incorporated into precisely designed delivery systems which fully exploit the potentialities of the drug for treatment of specific disease states (2, 3).

A major problem in the study of percutaneous absorption is the interpretation of results of previous investigators, since the permeability of mammalian skin has interested scientists from differing disciplines (4). It is difficult and dangerous to draw valid conclusions and comparisons due to the use of various test systems, *in vitro* and *in vivo* models, various times and modes of application of material, and several species from mice to humans. The results have been expressed in many different ways; each worker has been anxious to express the data so as to elucidate his or her own problem without making assumptions about the mechanism.

This review attempts to draw together the more significant work. No attempt is made at cataloging the extensive literature. Reference 1 is the authoritative treatise up to 1954, and several fine reviews are available (2–18).

METHODS FOR STUDYING PERCUTANEOUS ABSORPTION

Percutaneous absorption has been studied in many ways, but the varied techniques do not give comparable quantitative data. Few of them determine the avenues of penetration, and rarely is the ultimate deposition of the penetrating substance accurately made clear. Progress in the area of transport through the skin has been slowed by the absence of good methodology. Since the amount of penetrating material is so small, good quantitative microtechniques must be available. Often a physiological yardstick, such as vasoconstriction or vasodilatation, has been used to quantitate penetration. Limited availability of tissue for *in vitro* experiments also has slowed progress. Yet the techniques are improving and have yielded valuable data on the routes of absorption, the barriers to absorption, the degree of systemic absorption after topical application, and the effects of various physical and chemical parameters. Numerous methods for studying percutaneous absorption have appeared (1, 2, 5, 7, 9, 14, 19–37).

The methods can be divided into *in vitro* and *in vivo* procedures involving two general types of test systems. The former involves excised skin in diffusion chambers; the latter employs the skins of the living animal or human subject *in situ*. Some investigators have access to surgical skin specimens or to freshly obtained skin of human cadavers and can obtain epidermis with its complete stratum corneum by separation from the dermis.

Those who lack these sources resort to the pressure-sensitive tape-stripping technique for removing sheets of stratum corneum, of about 15 μm thickness, for use in diffusion cells (38). It is rarely possible to remove the stratum corneum entirely in this way, but most of this layer can be obtained. The stripping technique enables one to work with human skin membranes whose resistance to penetration closely resembles that of the entire thickness of the epidermis. Cantharadin-blistered skin may be used if it contains no holes. Indeed, the intactness of any skin preparation should be confirmed by microscopic examination prior to and after conducting a diffusion study.

The disadvantage of the excised skin technique is that it does not reflect the role of the skin *in vivo;* but if the main barrier to percutaneous absorption is viewed as a semipermeable membrane, then the results obtained are acceptable. When comparable *in vivo* and *in vitro* experiments have been performed, almost comparable results have been obtained (39–41). Scheuplein (42) observed that when the extant data on skin permeability are considered in the light of both steady-state and transient diffusion, much apparently conflicting data can be resolved. *In vitro* measurements of skin permeability are invariably measurements of steady-state rates if for no other reason than it is usually impossible to detect concentrations within the first few minutes.

Thus, previous measurements (25, 35, 43) are all of steady-state fluxes and are in agreement about the paramount role of bulk diffusion through the unbroken stratum corneum. On the other hand, *in vivo* permeability measurements are rapid measurements and often depend on biological end-points sensitive to extremely small concentrations (44–46). The *in vivo* results show only that shunt diffusion is predominant in the early transient stage of diffusion. This area will be explored more fully in the *Skin Transport* section.

The advantage of *in vitro* procedures is the absolute control obtained of environment, allowing the demonstration of the importance of individual factors in determining percutaneous absorption of a particular substance. These *in vitro* results may point the way for further *in vivo* work. If their outcome supports animal and human experimental findings, they may have a useful place in the screening of new products without the difficulties usually involved with *in vivo* methods. Marzulli *et al.* (26) sought to develop data through excised human skin in diffusion cells first and then to study skin absorption in human subjects.

The main objection to the use of the living animal or human subjects is that this method measures skin penetration by indirect means. For example, the test substance is applied to the skin and penetration is estimated by what is recovered in the urine. In such a system, it is not possible to find out to what extent the penetrant agent is metabolized as it traverses the skin en route to the bloodstream. Furthermore, a correction must be made for the storage of the penetrant in body organs. This storage correction is often obtained by measuring urinary recovery of the penetrant after introducing a known amount of agent by a single intravenous injection. Thus, the organ storage value may have a sizable error in cases where significant percutaneous penetration takes place over several days. These limitations suggest that there is room for progress in methodology. Moreover, diverse but complementary data may be obtained from both *in vitro* and *in vivo* studies (26).

***In Vitro* Methods**—*Excised Skin*—Virtually all investigators use some type of diffusion cell, in which

animal or human skin is fastened to a holder and the passage of compounds from the epidermal surface to a fluid bath is measured (47). The simplicity of equipment has prompted widespread use of the techniques. For example, Cronin and Stoughton (48) stretched human skin over the mouth of a funnel, and Fritsch and Stoughton (49) developed a special glass chamber which allowed various refinements to be employed. With this technique, penetration rates can be quantitated. The area of spread of a radioactive agent on the surface is detected with autoradiographs of the surface, and the penetration of an agent can be expressed in terms of quantity per unit area per unit time.

Radioactive agents have not taken over completely. Many chemical agents penetrate in sufficient concentration to be determined by one of the many techniques of physical or chemical analysis. One objection to this technique is that it does not represent the *in vivo* problem of an efficient, if capricious, blood supply which would remove penetrating materials at various rates and thus change the concentration gradients across the membrane or, as in the case of steroids, induce vasoconstriction (which occurs *in vivo*) and thereby decrease the rate of removal of steroid in the corium. This process would increase the local concentration in the corium and decrease the penetration rate. Thus, both *in vivo* and *in vitro* measurements for a given agent are highly desirable (23).

Penetration into Models—This method involves penetration of some substance into a model designed to simulate skin. If a chemical in a given vehicle is applied to the surface of a model, the amount of that chemical transferred from the vehicle into the model may be measured with a certain degree of accuracy. By using various vehicles, the rates of transfer of a given chemical into the model can be determined. The most common model used to simulate skin was agar gel, but the data obtained were very crude and more suitable models were sought.

Recently, in attempts to develop practical vehicles, models have been devised for studying *in vitro* release from the base into immiscible solvents, acting as "sinks" or receptor skin phases (50–54). Results have established fair correlation with membrane testing. These models are discussed more fully in a later section.

***In Vivo* Methods**—The major *in vivo* techniques involve histological studies, use of tracers, elicitation of biological responses after topical application, and analysis of body fluids or tissues.

Histological Studies—Tissue changes in skin following application of various substances to the cutaneous surface can yield information about the specific tissues affected by any penetrant, so that not only absorption *per se* is revealed but also the route of penetration. The method is limited to dyes and to a few other substances that yield precipitable colored end-products in specific chemical reactions. An objection to the method is that evidence of routes of absorption using colored dyes cannot be accepted completely, because a dyed lipid applied to the skin may not penetrate intact and, indeed, the dye may combine with another lipid in the dermis or epidermis. Separation within the skin of the dyed substance and the dye is generally the rule.

Histochemical techniques have proved useful in determining routes of absorption but are limited by the numbers of drugs influencing enzyme systems. For instance, changes in epidermal sulfhydryl groups are easily detected, but the number of drugs producing these changes is limited (55–58).

Tracers—Following the movement of penetrants by means of dyes, fluorescence, or radioactive labeling represents the most frequently used method. This technique is always combined with other methods such as histological or chemical analysis of tissues or fluids.

The dye technique employs an oil-soluble dye to color the material being studied. Histological sections of the skin are then examined for the presence of the dye. This method presupposes that the tracer and the penetrant act similarly and remain together during penetration. Unfortunately, this may not always be the case.

The use of fluorescence techniques depends on the fact that substances that penetrate very readily show a more rapid diminution in the intensity of the fluorescence at the surface of the skin than do poor penetrators. The use of fluorescent compounds in penetration studies is open to the same criticism as when a dye is used as a tracer. One cannot be sure that the fluorescent material remains with the substance whose penetration is being studied. This objection is not valid if the penetrating substance itself is fluorescent. If used properly, the method is of considerable value, as when Baker and Kligman (59) used the fluorescent dye tetrachlorosalicylanilide to determine the permeability of the horny layer.

Radioactively labeled tracer compounds gave the greatest impetus to skin penetration studies. Radioisotopes have been used to study percutaneous absorption by measuring the urinary excretion of topically applied compounds (60–69), the decrease in the radioactivity of the skin surface (28, 70), or the accumulation of an isotope in the collecting fluid of a diffusion or continuous-flow cell in which a piece of excised skin was mounted (35, 43, 62, 71). The method of measuring the rate of disappearance from the skin requires much smaller amounts of radioactive material than does the determination by blood analysis of how much drug has passed into the circulation.

While the methods give information on the amount of the compound that moves across the skin, they yield little or no information on the route of penetration or on the localization of the penetrant within the structure of the skin. Actually, all work with radioisotopic compounds should be checked at some stage by chemical recognition of the compound concerned, because enzymatic splitting of the molecule may occur and the radioactivity counted may not represent the material under investigation.

Marzulli *et al.* (26), while studying the penetration of ^{14}C-testosterone, noted inconsistencies in permeability constants, which suggested that there was a tissue reaction. Such a reaction could affect the diffu-

sivity of the membrane or change the form in which testosterone penetrates the skin. The method also has the problem of a 5–15% error, which is almost impossible to avoid. Thus, it is difficult to measure penetration of a small percentage of applied material. ^{14}C-Agents pass into the horny layer where fewer emissions are recorded on the surface, giving a false impression of penetration.

If further information is desired about the routes of penetration, autoradiography is a useful procedure, but it is difficult to quantitate. The considerable scatter of the rays and showing are drawbacks (72). Some β-particle emitters darken areas up to 2–3 mm away from the localization of the molecule, giving one a mistaken impression of the location of the agent. Therefore, tritium-labeled agents are very useful because of their weak β emissions. Cutting, staining, and handling of tissue not only may displace agents but also may remove agents from the specimen, giving a false negative result.

Analysis of Body Fluids or Tissues—For body fluid or tissue analysis, urinary analysis is used most frequently and is probably the method most commonly used in studying percutaneous absorption. Small specimens of skin, obtained by biopsy either singly or in series, may be analyzed for the penetrant. In cases where drugs appear to have a greater affinity for a particular organ, the organ may be excised and analyzed for drug content. The chief analytical tool is radioactive labeling, followed by GC, fluorescence, and specific color reactions.

Feldmann and Maibach (63–65, 73–77) utilized urinary excretion for studying various compounds. The method is extremely valuable. However, although the chemical agent recovered in the urine will probably have penetrated the skin, the amount recovered in the urine will not necessarily be the original total amount to penetrate the skin. Some of the applied agent may go somewhere other than the urine, and some may be metabolized and, therefore, no longer be detectable. A steady state of balanced absorption and excretion needs to be reached before measurements can be accepted completely. This is time consuming and may need the resources of a metabolic ward. Measurement of the excretion rate of metabolites of labeled corticosteroids has been employed successfully (61, 70, 78). Another source of error in this method may be the ability of the living organism to alter the penetrant before it is measured quantitatively. When such alteration occurs, more of the chemical penetrates than can be retrieved by chemical analysis.

Under *in vivo* conditions, penetration often occurs from such a large amount of material, in comparison to the amount penetrating, that the concentration of the solute in the presenting material remains almost constant. Also, when the penetrating material reaches the bloodstream, it is probably removed so rapidly that the concentration in the bloodstream is always near zero. Hence, detection in blood is rarely attempted.

A useful modification involves "calibration" of the animal to be studied. Suppose that the blood is to be analyzed as a measure of penetration but that some material reaching the blood is known to be lost to other tissues. If the penetrant is administered slowly by the intravenous route and the level of this substance in the blood is determined at successive time intervals, a calibration curve can be constructed which relates the amount of penetrant introduced into the bloodstream to the amount of that substance actually present in the blood. If, subsequently, the penetrant is applied to a given area of the skin of this animal and the blood level of this substance is determined at successive intervals, the amount of penetrant reaching the blood *via* the percutaneous route can be determined from the calibration curve constructed from data obtained after intravenous administration. With a little experimentation, the rate of change in the blood level can be made quite similar for the two methods of administration. This method is probably one of the best available for quantitative measurement of percutaneous absorption in the living animal (21, 79).

Elicitation of Biological Responses—The use of agents that have biological activity when they reach the corium is a valuable method. With this technique, it is possible to demonstrate whether or not a substance capable of causing a physiological reaction can penetrate into the skin and, if it does penetrate, to determine the time required for the reaction to develop. The method is intriguing because of its simplicity and potential practical applications. In theory, such biological functions of skin as sebaceous gland growth and excretion, sweat gland secretion, pigment formation, vasoconstriction, vasodilation, vascular permeability, epidermal proliferation, and keratinization could be influenced by topical pharmacological agents.

Many responses (sweat secretion, vasoconstriction, vasodilation, pigmentation, and vascular permeability) can be reasonably accurately recorded by visual observation. Thus, when enough of a topically applied agent such as histamine reaches the vessels of the corium, there is a visible reaction. The amount needed to induce the reaction can be determined by intradermal injection of histamine in differing dilutions to find the minimum dilution necessary for a biological response (23). This same approach can be used for such drugs as norepinephrine, angiotensin, naphazoline, nicotinic acid (niacin), atropine, and glucocorticoids.

Quantitation of the actual amount of the agent penetrating is not as accurate as with *in vitro* methods, but comparative estimations of the penetration of a given agent in such varying environmental circumstances as humidity and temperature can be meaningful, and much information regarding comparative effectiveness of topically applied steroids has been derived from this approach (23). One sensitive test is based on the antigenic properties of the substance to be absorbed, because extremely minute traces penetrating to the site of tissue antibodies may elicit allergic reactions.

The elicitation of vasoconstriction or vasodilation by the topical application of various agents has been

successfully employed (44, 48, 49, 80). This technique led to the development of the vasoconstrictor assay for the efficacy of topical corticosteroids (81). The relative potency of various corticosteroids has been assayed with this *in vivo* technique. It seems to be one of the most valuable methods available for estimating clinical efficacy of various topical glucocorticoids. The most potent glucocorticoid will give blanching of the skin when applied topically in a concentration of less than 1 ppm. There is little doubt that a relationship exists between the ability of topical steroids to produce blanching and to alleviate inflammation in use (82).

A technique involving paired comparisons of vasoconstriction at adjacent sites was found to improve the assessment of relative vasoconstrictor activity (83). In addition to the vasoconstriction test, use is also made of the suppression of UV-induced inflammation, the suppression of chemical inflammation from croton oil, and the clinical suppression of psoriasis (84). The results obtained by the four test procedures not only correlate very well with each other but they are also in good accord with *in vitro* release data.

Vasoconstriction can be induced by topical application of other agents such as naphazoline, and this method has been used to study regional differences in penetration (85). Curtailment of sweat was blocked by topical applications of anticholinergic agents (86, 87). Vasodilation can be introduced by a number of drugs, including nicotinic acid and some of its derivatives. The amount per area necessary to give vasodilation with intradermal administration is compared to the amount per area to give vasodilation by topical application. A rough estimate can be made regarding the amount penetrating the skin (80). The elicitation of biological responses has permitted the construction of time–response curves, particularly those derived from the vasoconstrictor results in the evaluation of the absorption and activity of corticosteroids in an ethanol solution and in different bases (88).

Determination of the threshold concentrations of pharmacologically active substances, such as vasodilators and vasoconstrictors, that cause the skin to respond is possibly the easiest method, but it is indirect and not very precise. An important limitation is that the end-points express thresholds, not rates of penetration. Another drawback is that the method does not discriminate between the two major pathways a substance may take in entering the skin—*viz.*, *via* the hair follicles and sweat ducts (appendages), thus bypassing the horny layer, and *via* diffusion through the mass of the horny layer itself (59). The bioassay methods for measuring percutaneous absorption have been reviewed critically (89).

SKIN TRANSPORT

Skin is a structurally complex, thick membrane. Molecules moving from the environment across the intact skin of living humans must first penetrate the stratum corneum and any material of endogenous or exogenous origin on its surface. They must then penetrate the viable epidermis, the papillary dermis, and the capillary walls into the bloodstream or lymph channels, whereupon they are removed from the skin by the flow of blood or lymph. To move, molecules have to overcome a different resistance in each tissue. Transport across the skin membrane is obviously a complex phenomenon, challenging in analysis (10). To relate results on different penetrants, it is necessary to consider the transfer mechanism across skin. A recent symposium (90) was principally concerned with transport through the skin.

Epidermal Barrier—The currently most accepted theory of percutaneous absorption treats the skin as a uniform and invariant cellular matrix which produces a thin dead layer of impermeable membrane protecting the body (3). The surface lipid layer seems to offer relatively little resistance to the passage of water and other chemical compounds. The main barriers to absorption are the dead cells of the stratum corneum (horny layer), restricting the inward and outward movement of chemical substances and having a high electrical resistance (91, 92).

The stratum corneum is a heterogeneous tissue (93), composed of flattened keratinized cells. The outer layers of these cells are less densely packed than those adjacent to the underlying granular layer. Therefore, the epidermal barrier becomes more impermeable in the lower part, and this fact has led to the suggestion that a separate barrier exists at this level, the so-called stratum conjunctum (31, 94). No real evidence exists for the localization of the functional barrier. Analysis of penetration data, evidence from controlled stripping experiments, and the detailed picture of the stratum corneum, gained from electron microscopy, all support the idea that the barrier to penetration consists of the keratin–phospholipid complex in the dead and relatively dry cells of the entire stratum corneum (31, 35, 42, 93, 95–101).

Thus, as molecules move from the environment into the skin, the rate-limiting barrier, *i.e.*, the tissue that presents the greatest resistance to the movement of molecules, is the stratum corneum—the entire stratum corneum. One or two of the outermost cell layers may offer only limited resistance because the cells are desquamating and the layers are breaking up, but the rest of the stratum corneum may be considered relatively homogeneous as far as its resistance to the movement of molecules is concerned (102).

Kligman (93) showed that human stratum corneum can be removed intact in large sections in the form of a thin (10–15-μm) membrane and that this membrane is mechanically quite strong and resists chemical attack. The accessibility of stratum corneum and epidermal membranes makes possible the controlled physicochemical study of skin penetration *in vitro* (35).

Scheuplein (42) calculated the diffusion constants and geometrical data corresponding to appendages and to various regions near the surface of the skin. There are significant regional variations in thickness and number of cell layers as well as marked individu-

al variations within a region that are characteristic and specific for each individual. The number of cell layers appears to account for the variation in thickness (103).

Information is limited about the composition of the barrier. The main cellular components are proteins, lipids, and water, combined into an ordered structure (104). The cells are bound into a layered membrane, about 15 μm thick when dry but about 48 μm when fully hydrated (31). When hydrated, the stratum corneum contains approximately 75% water, 20% protein, and 5% lipid. While the surface lipids offer little resistance to the passage of compounds, studies of lipid removal from the cutaneous surface (105–110) indicate that lipids participate in epidermal water function.

Onken and Moyer (109) showed that the barrier function is restored when extracted lipids are returned to the skin. This finding suggests some possibility of marked variations in biological membrane permeability, dependent largely on the specific nature or distribution of the lipid contained in the cell membrane. Sweeney and Downing (110) recently applied a number of solvents to the epidermal side of hairless mice and human skin. All solvents increased the rate of diffusion of tritiated water, but there was no correlation between the type or amount of lipid extracted and the degree of alteration of the water barrier function.

The barrier to penetration by the horny layer holds for almost all compounds tested. For example, a 10% naphazoline concentration gives vasoconstriction when applied to normal skin, but a 0.001% concentration gives vasoconstriction when applied to skin where the horny layer has been removed by cellophane tape stripping (20). Skin with a disrupted epidermal barrier allows up to 80% of hydrocortisone to pass into the dermis. With a functionally intact epidermis, the absorption of steroid is about 1% (70).

The skin barrier is vulnerable to certain solvents and organic amines, as reflected in sharp increases in skin permeability (36). Solvents with such an effect are those with both polar and lipophilic properties. Apparently, they have the effect not only of removing skin lipids but also of breaking down the complex structure of the barrier, thus making it defective and possibly subject to entry by other chemical agents which do not penetrate intact skin. Substances such as dimethyl sulfoxide, dimethylformamide, and dimethylacetamide have the ability to produce a striking but reversible suppression of barrier resistance. It has been suggested that they alter the interrelationship of water, lipids, protein, and mucopolysaccharides regulating the epidermal water barrier function (112). The effects of these "accelerants" will be discussed more fully later.

Barrier Reservoir—The existence of a depot or "reservoir" within the stratum corneum was originally noted by Malkinson and Ferguson (60) and investigated in detail by Vickers (113). Vickers applied small quantities of fluorinated steroids to the surface of the skin and occluded the area with plastic film. As expected, vasoconstriction or blanching appeared when the film was removed after 16 hr (81). However, if the skin was then thoroughly washed and the subject was allowed to continue normal activity, the vasoconstriction faded in 10–16 hr. If areas were reoccluded at intervals as long as 12–14 days after the initial application of steroid, vasoconstriction reappeared, although no more steroid had been applied.

It was obvious, as a result of these observations, that a reservoir for steroids must exist somewhere in the skin. Clinical and radiobiological studies suggested strongly that the reservoir lay in the stratum corneum and that it was not just a surface film (114). It appears to lie in the deeper portions of the stratum corneum. The presence of an intact and normal stratum corneum is essential for the establishment of the reservoir (115). The amount of materials that can be retained within the reservoir is small, and their significance from a therapeutic point of view is open to question, although potent corticosteroids, such as fluocinolone, may be retained in sufficient quantity after a single application to influence epidermal cell division (116). Woodford and Barry (117) studied the bioavailability of betamethasone benzoate in a cream and gel base as well as other steroids. All formulations produced steroid retention in the stratum corneum for at least 8 days.

Garnier (88) discussed the parameters to be considered in devising the optimum base for topical preparations of corticosteroids. When the activity of the drug, as with steroids, depends on absorption into the vascular system in the dermis, the base may need to lower the interfacial barrier if the epidermis is intact or soften the superficial tissue if it has become excessively dry and thick.

Various factors that increase percutaneous absorption also appear to increase the quantity of material in the reservoir. Increasing temperature and humidity (49) result in increased penetration and in the establishment of a larger stratum corneum reservoir (113). Similarly, a solvent such as dimethyl sulfoxide enhances human skin penetration (118) and increases the reservoir (111). Dimethylacetamide and dimethylformamide are potent solvents which have been shown to increase the penetration of various steroids and griseofulvin and to establish, both *in vivo* and *in vitro*, a stratum corneum reservoir to a superior degree than such vehicles as ethanol or cream base (119).

Epidermal Diffusion—An understanding of the diffusion mechanism through skin may have even wider significance to the mechanism of membrane transport in general. The stratum corneum is an aggregate of tightly adherent cells, densely packed with lipid-enriched keratin fibrils, and presents a unique system for studying the chemical influence of naturally occurring protein–lipids on membrane transport. This is particularly significant when it is realized that many cell membranes are chemically quite similar to the stratum corneum and that transport through them is believed to be governed mainly by a lipid–penetrant interaction. In this restricted sense, the stratum corneum is a macroscopic analog of the unit-cellular membrane (35). Poulsen (120) reviewed

aspects of drug diffusion into skin.

Diffusion through the horny layer is a purely passive process, affected only by physical factors as determined by ambient conditions. Percutaneous absorption, on the other hand, is a more complicated process; epidermal diffusion is the first phase, and clearance from the dermis is the second. The latter depends on effective blood flow, interstitial fluid movement, lymphatics, and perhaps other factors such as combination with dermal constituents (35). A passive system has two main characteristics: *(a)* a delay period after the chemical is placed on the surface, during which the membrane itself becomes charged with the penetrant, and *(b)* a steady penetration rate after the delay period, which lasts as long as the chemical remains in adequate supply on the top surface and is removed from the lower surface. This steady rate is proportional to the concentration difference across the membrane; in the case of adequately perfused skin, the rate may be considered equal to the concentration applied (91). The ratio of the steady rate to the concentration applied should be, and is, a constant (termed the permeability constant); it is a measure of the permeability of the given skin to the chemical in the given solvent. If, and only if, results are expressed in terms of this parameter, they can be compared directly with each other (4). Physicochemical relationships involved in skin penetration have been derived (10, 21, 22, 31, 35, 43, 98, 102, 107, 108, 121–129).

There is little evidence to support specialized active transport systems for cells of the stratum corneum, except possibly for the work with water[1]. For water to move against a concentration gradient, an energy yielding "pump" is predicated in a viable epidermis. It is unlikely that such a reaction would exist in a nonviable tissue like the stratum corneum, and there is limited evidence for any transport across the stratum corneum against a concentration gradient. While the preponderence of evidence points to passive diffusion through a nonviable horny layer, Winkelman (90) was concerned that this assumption discounts the many rich variations of epithelial and mesenchymal structure which appear to have some role in the passage of material through the skin.

ROUTES OF SKIN PENETRATION

There are potentially three distinct routes of penetration through the stratum corneum: *(a)* the follicular regions, *(b)* the sweat ducts, and *(c)* the unbroken stratum corneum in between. But which is the principal route of penetration? There is little convincing evidence that eccrine sweat glands or ducts play any significant role in cutaneous permeability (5, 30). Material may enter the ducts and even the glands, but there appears to be no subsequent penetration to the dermis (130–132). Absorption of allergens and histamine through the palms and soles is very poor (44, 133). These are areas where, in view of the nature of the keratin, transepidermal absorption would be minimal; any absorption taking place must do so through the sweat glands because pilosebaceous glands are almost absent.

Hence, percutaneous absorption occurs through the cells of the stratum corneum, between the cells or *via* the pilosebaceous follicles. The particular route may be of major importance for one type of substance, but both routes probably play some part in the absorption of almost all compounds and the relative importance of one in contrast to the other depends almost entirely upon the physicochemical characteristics of the material being absorbed (2). In spite of specific literature statements, there are many difficulties in deciding the relative importance of each route in any one instance; material using the transfollicular route may diffuse in the dermis and appear in the subepidermal tissues, making it impossible to determine the route used. When considering skin penetration, it is helpful to keep in mind the following particularly relevant dimensions based upon reported values: thickness of stratum corneum, 14 μm; thickness of epidermis, 110 μm; and distance from surface to microcirculation, 150–200 μm (42).

For substances absorbed by the transepidermal route, penetration is fairly rapid, although slower than intestinal tract absorption, and is almost always accompanied by some degree of pilosebaceous penetration. For substances absorbed through both pathways, the transepidermal route is the principal portal of entry because of the total relatively small absorbing surface offered by the pilosebaceous units (5). The epidermis presents a surface area 100 or 1000 times greater than the other routes of absorption. The sweat glands and hair follicles are scattered throughout the skin in varying numbers but are comparatively sparse; their total cross-sectional area is probably between 0.1 and 1.0% of the area of the skin. The total volume available for transport, other than within the stratum corneum cells, is probably 0.01–0.1% of the volume of the stratum corneum. The evidence supports the belief that transient penetration occurs *via* the shunts and that steady-state transport occurs directly through the cells.

Drugs applied to the skin surface reach the orifices of the sweat glands and the hair follicles directly. Each hair follicle has one or more connecting sebaceous glands which empties its secretion into the follicular canal near the skin surface. These canals and duct systems are lined with stratified squamous epithelium, which is readily penetrated by medicaments. Therefore, the most likely pathway for drug penetration *via* the transfollicular route is through the microscopic spaces between the hair shaft and the follicular wall. These spaces permit the passage of substances into areas below the membrane barrier. Hence, the hair follicles were formerly thought to be the main route of percutaneous absorption. They may play a more conspicuous part in the penetration of particular substances, notably surfactants, which provide an increased wetting effect and allow close contact between incorporated substances and the follicular epithelium.

[1] See K. J. Buettner and F. F. Holmes, *J. Appl. Physiol.*, 14, 261, 276(1958).

Diffusion through the stratum corneum occurs with considerable difficulty even for small, soluble molecules (7). Steady-state penetration through the stratum corneum is not primarily intercellular or intraappendageal. The hydrated stratum corneum seems best described as a dense "semisolid" phase into which low molecular weight nonelectrolytes dissolve with strong chemical interaction and through which diffusion occurs very slowly. Before steady-state diffusion is established, small but possibly significant amounts of material may diffuse more rapidly through hair follicles and sweat ducts. The reasons for this seemingly anomalous behavior arise from the limited area of the skin surface occupied by these appendages, their relatively large diffusion constants, and the nonlinear character of diffusion prior to the steady state. The smaller the diffusion constant, the more important the role of the appendages or intercellular regions may become (124). Shunt diffusion may become dominant and control percutaneous absorption not only in the transient period but during steady state as well.

For molecules such as the steroids, which move very slowly through the stratum corneum and have long lag periods, the shunts may at all times be the major pathway into the skin (124, 134). Disease states, particularly those that affect the stratum corneum such as eczema and exfoliative dermatitis, undoubtedly result in many "artificial shunts" through the stratum corneum. Body fluids escape through such shunts, and topically applied medicaments can enter more easily through them than through normal skin (10).

In a study (43) of steady-state diffusion of alcohols from aqueous solutions, the high molecular weight, lipid-soluble alcohols had larger permeability constants than the low molecular weight, water-soluble alcohols. This finding indicates that the mechanism of steady-state diffusion of each of these alcohols cannot be only diffusion through relatively large, water-filled channels. If this were the mechanism, the permeability constant would change very little over the entire series; actually it would decrease slightly with increasing molecular weight. Low molecular weight molecules readily penetrate capillary walls and rapidly enter the systemic circulation. The rich vascularity of the superficial layers of the dermis limits the number of molecules free to diffuse deeper into the dermis, so lower lying layers of the dermis would not be expected to influence diffusion radically (42).

Polar and nonpolar substances diffuse through the stratum corneum by different molecular mechanisms (125). As the stratum corneum hydrates, water accumulates near the outer surface of the protein filaments. Polar molecules appear to pass through this immobilized water. In contrast, nonpolar molecules probably dissolve in, and diffuse through, the nonaqueous lipid matrix between the protein filaments. The activation energies for the diffusion of nonpolar molecules are somewhat lower than for the diffusion of polar molecules.

Scheuplein (42) maintained that it is probably wrong to assume that a principal route of penetration exists without specifying other conditions. Under the appropriate conditions, each contending route of permeability may be, in turn, overwhelmingly dominant. In particular, the transient diffusion occurring shortly after the application of a substance to the surface of the skin is potentially far greater through the appendages than through the matrix of the stratum corneum. After steady-state diffusion is established, the dominant diffusion mode is probably no longer intraappendageal but occurs through the matrix of the stratum corneum. Flux through shunts is difficult to measure experimentally, except possibly through hair. The recognition of transient diffusion occurring primarily *via* follicles and ducts and steady-state diffusion primarily through the intact stratum corneum results in a considerably more self-consistent and orderly treatment of the process of percutaneous absorption.

The role of intercellular spaces in cutaneous permeability has been pondered (43), but no one has seriously proposed these canals as an avenue for percolation of materials through the epidermis. The total volume of the intercellular space has been estimated to be 0.01–0.1% of the entire volume of the stratum corneum. Substances would have to move rapidly through the small intercellular space if this pathway were to contribute appreciably to the steady-state flux (53, 90). Few studies of the significance of this pathway have been attempted. It has been assumed that the principal route of entry is directly through cells rather than between them.

Once a substance passes through the superficial barrier, there is apparently no significant hindrance to penetration of the remaining epidermal layers and corium, following which there is ready entry into the circulation *via* the capillaries. However, for certain materials there may be a second barrier to absorption at, or near, the dermal–epidermal junction and possibly in relation to the basement membrane (38, 132, 135–137). A salient example of this barrier action, demonstrable with autoradiographs, is the almost complete arrest at the dermal–epidermal junction of certain electrolytes once the superficial barrier is passed (130–132). Thorium-X and ^{32}P-labeled sodium phosphate applied to the skin surface cannot be shown to be present in the dermis on autoradiographs, despite the fact that penetration into the epidermis and follicles occurs (132). There may be a double retention effect, one in the epidermis and a second in the dermis.

Menczel and Maibach (136, 138) recently noted considerable retention of testosterone in the dermis. While the role of dermal retention needs closer examination, the superficial barrier is the critical one for most substances; once it is breached, further passage into the epidermis, corium, and capillaries is virtually assured. The stratum corneum, the epidermis, and the uppermost part of the papillary layer of the dermis (*i.e.*, the portion subjacent to the basal layer of the epidermis and above the capillaries of the microcirculation) are together assumed to comprise the effective composite layer through which substances must diffuse to enter the bloodstream (42).

The factors that influence the penetration of the skin barrier are essentially the same as those that influence GI absorption. These factors can be divided into physiological and physicochemical variables (37, 139, 140). Additional variables are the condition of the skin, the skin age, the area of skin treated, the thickness of the skin barrier phase, the species variation, and the skin moisture content. The rate of diffusion of substances through the skin is dependent primarily on the physicochemical properties of the drug and only secondarily on the vehicle, pH, concentration, *etc.* The initial discussion will deal with physiological skin factors; physicochemical factor considerations will follow.

Skin Condition—"Intactness" of the skin is one of the most important factors preventing penetration. Injurious agents such as mustard gas, acids, and alkalies injure barrier cells and increase permeability. When the stratum corneum is damaged, diffusive water loss is increased. This has been amply demonstrated in experimental injury to the stratum corneum by cellophane tape stripping (97). Complete removal of the barrier by stripping enhances the absorption of almost any substance in contact with the skin surface. In human subjects, 78–90% of hydrocortisone-^{14}C penetrated stripped skin sites (70) while only 1–2% was absorbed from areas of normal skin (60, 141). Loeffler and Thomas (142) found that 50% of ^{89}Sr-labeled strontium chloride was absorbed through abraded rat skin in contrast to 10% absorption in intact skin sites. In diseases characterized by a defective horny layer, percutaneous absorption is also increased (5, 8, 43, 100, 143, 144).

Solvents other than water appear to cause marked alteration in the resistance of the skin barrier to penetration (108, 118, 121, 145, 146). The permeability of the skin to aqueous solutes, organic liquids, and solutes in organic liquids and solids has been reviewed (4). Pretreatment of the skin with organic solvents has variable effects on permeability. Treatment with ether did not alter the penetration rate of salicylates or surfactants (145), while the polar solvents acetone, alcohol, and hexane greatly increased the penetration of water into the skin (109). Excised stratum corneum is virtually "opened" by delipidization of the stratum corneum by holding it in a mixture of a polar and a nonpolar solvent such as chloroform–methanol. This procedure removes much of the lipid fraction of the stratum corneum and makes "holes" or artificial shunts in the membrane (10). Measurements of the activation energies show them to be comparable to diffusion through water-filled channels in delipidized membranes.

Allenby *et al.* (147), using electric impedance as their parameter, studied the permeability of excised human skin to various solvents. Some organic solvents including aliphatic acids, bases, and neutral compounds were shown to produce large changes in electrical impedance and, it is inferred, in water permeability. Dimethyl sulfoxide, dimethylformamide, and dimethylacetamide produced large and rapid changes. The changes in water permeability, which are inferred from the impedance changes and which are induced by the various organic solvents, are probably due to a combination of factors including solution of skin components, relaxation of the binding forces of the skin components, and swelling and hydration of the skin structure with the formation of additional channels for water permeation (147).

The role of sorption promoters such as dimethyl sulfoxide (77, 118, 148) and surfactants (41, 149–152) will be more fully discussed in a later section.

Skin Age—The relationship of age to skin permeability has been rarely investigated. Fetal and infant skin appears more permeable than adult skin (73). Percutaneous absorption of topical steroids occurs more readily in children than adults (153). The significant dermal atrophy and gross epidermal changes in the elderly denote absorption influence (2). The epidermis of the preterm infant has a higher permeability than that of older infants or has a less complete barrier function (154).

Increased Blood Flow—If blood flow through the dermal vessels increases, the rate of clearance of materials should also increase as the concentration decreases. This is particularly true of gas permeation (3). Whether the rate of passage through the barrier layer is altered is not quite so clear, although it seems possible that this may occur because the more rapid removal of material that has penetrated must alter the perfusion gradient across this area. Clinically, erythematous skin is usually diseased, and this condition may alter the rate of absorption for other reasons (2).

If one considers the opposite state of affairs, however, then the evidence is more clearcut. Vasoconstriction produced by the application of topical steroids, a process that it seems hard to believe would damage epidermal cells, undoubtedly slows penetration. The induction of vasoconstriction by the topical application of 6-methylprednisolone slowed the absorption of ^{14}C-testosterone applied after the vasoconstriction had appeared (70).

Regional Skin Sites—There are relatively few studies on the variations in absorption from one site to another and many are conflicting, with variable epidermal cell counts and histochemistry (29–36, 38–41, 131, 155, 156). In different normal individuals, there are wide variations in the absorption rate of a given substance through the same skin site, and penetration rates for the most permeable regions (posterior auricular skin) in some subjects are comparable to rates for the least permeable regions (plantar skin) in others (38). Anatomical differences in penetration rates may depend largely on differing thicknesses of the barrier layer, since equally thick layers of stratum corneum conjunctum from plantar skin and from the anterior forearm, for example, show equal resistance to penetration (38).

Variations in penetration rates have been demonstrated for full thickness cadaver skin, isolated from different sites, with rates increasing in the following anatomical order: plantar, anterior forearm, instep, scalp and ventral thigh, scrotum, and posterior auric-

ular (38). These permeation rates may be in direct proportion to the thickness of the area, because the penetration across skin, the flux, is inversely proportional to the thickness. The thickness of the stratum corneum from pieces of glabrous skin can be calculated from data obtained from desorption experiments (128).

The thickness of thick pieces of cornified epithelium (callus) can be measured directly, and the flux of water through these pieces can be determined (129). Fick's law, treated in a later section, governs passive diffusion and can be expressed as:

$$J_s = \frac{D_m}{\delta} \Delta C_s \qquad \text{(Eq. 1)}$$

where δ is the thickness of the stratum corneum, and D_m is the diffusion constant. The flux is proportional to the concentration gradient, ΔC_s, *i.e.*, the difference in concentration divided by the thickness.

Cronin and Stoughton (85), using the erythema reaction produced by vasodilators such as ethyl nicotinate and histamine, demonstrated that the forehead, presternal area, and back showed a greater response than the limbs and that the arm was more reactive than the leg. They concluded that the presence of more follicles in the forehead indicated increased penetration through sebaceous glands. In an earlier study with topical histamine, Shelley and Melton (44) did not observe any difference in urticaria among the arms, back, chest, thigh, and leg. Feldmann and Maibach (75), in studies of ^{14}C-hydrocortisone, noted that measurable absorption occurred through all regions of human skin except the heel. The absorption seemed greater in areas where follicles were large or more numerous, such as the forehead and scalp, and decreased where the stratum corneum was thicker, such as the foot.

Tregear (157), on the other hand, from studies of rapidly absorbed tributyl phosphate, concluded that hair follicles do not increase penetration. Laug *et al.* (158), working with rabbits, studied the penetration of mercury ointment through the skin and found no significant difference in penetration between the back and the belly. However, Smith *et al.* (155) observed a difference between the times taken for local anesthesia to develop on scrotal and abdominal skin after the topical application of lidocaine. With *in vitro* experiments, they also found other differences in the penetrability of skin between these two areas.

Species Variation—Humans and animals display wide differences in physical characteristics such as the number of appendageal openings per unit area and the thickness of the stratum corneum. These physical and structural differences obviously affect the penetration pathways and the penetration resistance of skin (26). The skin of rabbits, rats, and mice, which have been used frequently in work on percutaneous absorption, lacks sweat glands and abounds in hair and hair follicles in contrast to human skin. In addition, it is virtually impossible to apply small amounts of aqueous solution uniformly to hair-clipped animal skin. Moreover, when animals are used, some additional technical care must be taken to avoid unexpected disturbance of that part of the skin where a sample has been topically applied.

In spite of all these limitations, animals have been used in many studies on the percutaneous absorption of various substances, because biologically dangerous substances, including radioactive compounds, are under many restrictions as to the application to human subjects and the excision of normal human skin for the evaluation of any substance absorbed through the skin is hard to accomplish (44, 159–161). Yet, human skin, either living or excised, is easily obtained and usually more suitable than animal skin if the intent is to find out about the penetration capacity of a substance applied topically. Furthermore, biochemical differences between animal and human skin, even when subtle, may significantly alter skin reactions with penetrant chemicals (26).

Species variations of barrier permeability have been noted only in the broadest sense (3). The skin of common laboratory rodents is more permeable than human skin (4). The relationship between species is not consistent for different substances, but the average permeability order is rabbit > rat > guinea pig > humans. The largest deviation from the average is that human skin is very much less permeable to ions than is rabbit or guinea pig skin. While penetrability through rabbit skin is rapid, the structure of the epidermis or appendages does not appear to differ significantly from that of other animals more resistant to penetration (3, 162, 163). In certain studies, intact guinea pig skin has given results similar to human skin (164).

Notable studies include those of Stolar *et al.* (165), who used rabbits to study the absorption of sodium salicylate from hydrophilic ointment. Desgroseilliers *et al.* (69) used young domestic pigs for topical steroid absorption. Sweeney *et al.* (146) chose hairless mice for an investigation of the effects of dimethyl sulfoxide on water passage because of the absence of eccrine sweat glands and the presence of small-sized hair follicles. Penetration of fluorouracil through human and hairless mouse skin was measured *in vitro*, and the results were compared using radioactive-labeled drugs and a GC method specific for the free fluorouracil molecule (166). Significantly greater drug penetration was obtained in the mouse samples using both methods of detection.

Hydration—Hydration of the stratum corneum is among the most important factors in skin penetration, increasing the rate of passage of all substances that penetrate the skin. Hydration results from water diffusing from underlying epidermal layers or from perspiration accumulating after application of an occlusive vehicle or covering on the surface. Under occlusive conditions, the stratum corneum is changed from a tissue that normally contains very little water (5–15%) to one that may contain as much as 50% water. Permeability increases four- to fivefold.

Hydration apparently "opens" the compact substance of the stratum corneum, as can be demonstrated dramatically in rats by comparing the LD_{50} of percutaneously administered substances under oc-

cluded and unoccluded conditions. Brown (167) observed that the percutaneous LD_{50} of dinitrobutylphenol is 140 mg/kg when applied under occluded conditions and 1000 mg/kg under nonoccluded conditions. However, not all substances are influenced in the same way, and the same investigator found that the percutaneous LD_{50} of parathion was greater under occluded conditions than when the treated area was left uncovered.

Hydration of the epidermis has been shown to increase the percutaneous absorption of nicotinic acid (85) and salicylic acid (5). Wurster and Kramer (105) measured the rate of penetration of esters of salicylic acid through skin with dry and hydrated stratum corneum. When the tissue was hydrated, the rate of penetration of the most water-soluble ester increased more than that of the other esters studied. Working with aspirin in a temperature–humidity chamber, Fritsch and Stoughton (49, 118) showed the dual importance of these factors on the penetration of excised skin. Full hydration of the keratin, accomplished by layering water over ^{14}C-aspirin on the epidermal surface, dramatically increased the penetration when compared to conditions of 88% humidity at the same temperature. There is little doubt that heavy hydration of the horny layer drastically reduces the barrier efficacy.

Further evidence of the importance of hydration can be found in investigations employing occlusive plastic films in steroid therapy. Here the prevention of water loss from the stratum corneum and the subsequent increased water concentration in this skin layer apparently enhance the penetration of the steroid (81, 113, 168–170). McKenzie and Stoughton (81) showed that penetration of corticosteroids may be increased 100-fold by occluding the site of application and thus hydrating the stratum corneum. Vickers (113) demonstrated that occlusion not only enhances penetration of corticosteroids but also creates a depot effect in the stratum corneum.

The mechanisms of skin transport have already been discussed. The mechanism of transport of a drug through hydrated stratum corneum may be quite different from that through normal stratum corneum. Shelmire (171) suggested that the mechanism of hydration was to increase the size of the pores. There is not only a physical alteration of the tissue due to hydration, but at high water activities there are also changes in both the diffusion coefficient and activity coefficients of the penetrating agent (121). The important thing is the thermodynamic activity of water in the barrier phase, not just the amount there.

One would expect the rate of penetration of water-soluble drugs to be faster through hydrated than through normal stratum corneum (31). The low diffusion constant and high activation energy obtained for water and polar alcohols and the selective diffusion exhibited by molecules of varying polar character suggest that extensive hydration does not drastically affect the barrier function of the stratum corneum. The hydrated stratum corneum is one of the most water-impermeable biological membranes found in nature, although it is slightly more efficient before extensive hydration and presumably *in vivo* (35). A two-phase series model for the permeability behavior of the fully hydrated stratum corneum showed reasonable correlation between experimental permeability coefficients and partition coefficients (172).

Temperature—Relatively little attention has been paid to the effects of temperature on percutaneous absorption. Under normal *in vivo* conditions, substances penetrate the skin only within a very narrow temperature range. Clinical variations derive chiefly from occlusion. *In vitro* experiments may be conducted over a much wider range.

Blank and Scheuplein (31) observed little alteration of the permeability of the barrier from exposure for several hours to temperatures as high as 60°. However, Allenby *et al.* (147) showed that the stratum corneum undergoes irreversible structural changes when heated above 65° or when incubated in aqueous media at pH <3 or >9.

The dual effects of both temperature and humidity were shown clearly by Stoughton and Fritsch (118), who demonstrated considerable increases in the percutaneous absorption of aspirin and corticosteroids as the temperature rose and, especially, as humidity increased. *In vivo* work with occlusion (81, 113) and clinical studies (169) have corroborated the roles of temperature and humidity.

Blank and Scheuplein (31) studied the rates of penetration of ethanol and 1-pentanol within the 0–55° range. The flux, or the amount of alcohol penetrating per unit area in unit time, was an exponential function of the temperature. The energies of activation were determined by Arrhenius plots of the log of the permeability constant against the reciprocal of the temperature. The activation energies of the two alcohols differed measurably from one another and were higher than those obtained for diffusion of the same substances in solutions. The magnitude of the activation energies for the penetration of the low molecular weight alcohols through the skin indicated that penetration was a more complex process than diffusion through vehicle-filled channels.

Drug Concentration—The amount of drug percutaneously absorbed per unit surface area per time interval increases as the concentration of the drug in the vehicle is increased. Also, more drug is absorbed per time interval at a constant drug concentration if the drug is applied to a larger surface area. However, with a few compounds, increasing concentrations produce significant decreases in absorption rates. This finding has been recorded for substances that produce caustic effects on the skin in relatively high concentrations, thereby producing an artificial barrier—often with obvious crust formation—which impedes effective penetration. Such effects for substances that freely penetrate the barrier in low concentrations have been described for higher concentrations of phenol (173) and hydrogen sulfide gas (174).

The duration of contact with the skin as well as the concentration of the penetrant play important roles in skin diffusion. Tregear (4) showed that the perme-

ability constant of thioglycollic acid is dependent on concentration and probably on time of contact as well. The positive penetrative effects of increased concentrations of the steroids flucloronide (175), betamethasone (52), cortisone (76), hydrocortisone (76), testosterone (76), and androstenedione (76) have been demonstrated. In addition to the steroids, Maibach and Feldmann (76) showed penetration–concentration dependence for caffeine, salicylic acid, and benzoic acid.

Skog and Wahlberg (176) showed a definite increase in the absorption of various compounds with increasing concentration in guinea pigs. They noted increasing penetration up to a certain point, at which a plateau was reached. This finding may indicate that the barrier layer may not be influenced by diffusion gradients and may merely act as an absolute limiting step, limiting the total amount of any substance passing through in unit time. With true steady-state diffusion, permeability constants are independent of concentration. The neurological difficulties experienced in rats with topical administration of hexachlorophene prompted a series of investigations regarding its percutaneous absorption (177–181). Permeability constants, diffusion constants, and membrane–vehicle partition coefficients were determined (181).

One of the anomalies of the action of dimethyl sulfoxide on skin penetration is its unusual concentration dependence. Low concentrations are virtually without effect; as the concentration is increased, there is a rapid enhancement of percutaneous penetration (182). A direct relationship was obtained between the concentration of dimethyl sulfoxide and the rate of penetration of potassium methyl sulfate (183).

The effect of concentration as a factor controlling the rate of permeation of a drug through a membrane can be examined by reference to Fick's general law of diffusion which, in essence, states that the driving force causing the transfer of a substance from regions of high to regions of low concentration is proportional to the concentration gradient. Fick's law is commonly written as $J_s = K_p \Delta C_s$, where J_s is the flux across the membrane, K_p is the permeability constant, and ΔC_s is the difference in concentration on the two sides of the membrane.

By varying the concentration of the penetrant and observing the consistency of K_p, one can determine the extent to which Fick's law is applicable under the experimental conditions. When Fick's law is indeed applicable, the K_p value provides a satisfactory basis for comparing penetration rates obtained in different laboratories with different concentrations and techniques.

Over a wide concentration range, flux will be proportional to concentration only if the membrane remains unaffected by the concentrated solutions and if the partition coefficient remains constant over the entire concentration range (10, 91, 126). In a study of the penetration of low molecular weight alcohols (43), Fick's law held accurately only for relatively low concentrations. With high concentrations, the flux of some alcohols leveled off and then decreased.

Table I—Rating Chart for Human Skin Penetrants[a]

Penetrant Rating	Permeability Constant, μcm/min	Example
Very slow	<0.1	Aluminum salts
Slow	0.1–1.0	Tripropyl and tributyl phosphates, methyl-salicylate
Moderate	1.0–10.0	Trimethyl and triethyl phosphates, ethanol
Fast	10.0–100.0	*n*-Butanol, laurate
Very fast	>100.0	*n*-Pentanol

[a] From Ref. 26.

Marzulli *et al.* (26) proposed a scheme for rating substances as to their capacity for penetrating human skin according to their K_p value (Table I). Table I is based on permeability constants, most of which were compiled by Tregear (91) and which range from 0.02 to 200. Five categories are provided covering five orders of magnitude, with descriptive terms assigned to each category.

Solubility Characteristics of Penetrant—The solubility characteristics of a substance greatly influence its ability to penetrate biological membranes. The lipid–water solubility pattern of the applied material was recognized at the beginning of this century in the Meyer–Overton theory of absorption. This theory stated that, because the epidermal cell membrane consists of a mosaic pattern of lipid and protein molecules, substances soluble in lipids pass through the cell membrane owing to its lipid content while water-soluble substances pass after the hydration of the protein particles in the cell wall, which leaves the cell permeable to water-soluble substances. The validity of this theory and the importance of ether–water solubility coefficients have been shown on many occasions and in relation to many drug groups (2). In essence, the aqueous solubility of a drug determines the concentration presented to the absorption site, and the partition coefficient strongly influences the rate of transport across the absorption site (184).

Since the Meyer–Overton theory appeared, numerous papers have been published reporting the percutaneous absorption of various lipid-soluble substances. Since Minato *et al.* (160) comprehensively reviewed these studies, only recent notable studies will be included here. Tregear (4) reviewed the permeability of the skin to water, electrolytes, and organic solvents. Treherne (25) related the permeability constants of a series of compounds to their ether–water partition coefficients and suggested that a partition coefficient of unity might favor skin penetration.

A similar relationship between the vasodilator activity and lipid–water partition coefficient was demonstrated for a series of esters of nicotinic acid by Stoughton *et al.* (80). They also found a similar correlation between the benzene–water partition coefficients and the penetration of the epidermis by a series of closely related boronic acid derivatives. Cronin and Stoughton (48) estimated the penetration of nic-

otinic acid and one of its esters, ethyl nicotinate. They were able to show a very dramatic difference (37,000-fold) between the penetration rates of the two materials and postulated that the differing ether–water partition coefficients of the two compounds were at least in part responsible for the difference. They noted that ethyl nicotinate was some 10,000 times more volatile than nicotinic acid; since it is known that gases penetrate the epidermis very readily (185, 186), they postulated that the increased volatility was primarily responsible for the increased penetration.

The magnitude of the effect of moisture on the percutaneous diffusional rates of several salicylate esters has been shown to be proportional to the oil–water distribution coefficient and the water solubility of these closely related compounds (105). In the case of surfactants, the combination of hydrophobic and hydrophilic characteristics affect penetration (145, 183). Absorption profiles for a series of salicylates through intact rat skin showed decreases with increasing pH (187). Absorption apparently ceased if the degree of ionization was higher than 99.9% or if less than 0.1% of the drug was in the unionized form. For salicylic and *p*-aminosalicylic acids, absorption curves strictly followed the pH–partition hypothesis.

In the formulation of preparations for topical application, it is profitable to select or prepare compounds having the required solubility characteristics before attempting to promote their penetration by pharmaceutical manipulation. This is exemplified by the development of topical corticosteroid preparations. Triamcinolone possesses five times the systemic activity of hydrocortisone but only one-tenth of its topical activity. Conversion of triamcinolone to its acetonide enhances its topical activity 1000-fold (188). Similarly, betamethasone has 30 times the systemic activity of hydrocortisone but only 10 times its topical activity.

Of 23 esters of betamethasone examined (114), betamethasone 17-valerate possessed the highest topical activity and this coincided with the most balanced lipid–water solubility coefficient. The activity became less as the derivatives became more lipid and less water soluble. Compounds that were more soluble in water and less soluble in lipids were similarly less active on topical application. Katz and Shaikh (184), utilizing the vasoconstriction end-point for topical corticosteroids, secured results which seem to indicate that the efficiency of percutaneous absorption may be a function of the product of the partition coefficient and the square root of the aqueous solubility. The results are in agreement with developed theoretical considerations (121). This finding suggests that increases in topical corticosteroid anti-inflammatory activity, produced by molecular modifications, are in great measure proportional to changes in solubility and the partition coefficient. This effect of steroid solubility is a major factor in penetration from varied vehicles (51, 53, 54, 184, 189). Several cream and ointment vehicles were developed for flucloronide. Enhanced drug release and superior vasoconstrictor activity were demonstrated from vehicles where the drug was entirely solubilized (190).

Allenby *et al.* (147), in a study of the effect of various solvents on the electrical impedance of skin, found that dimethyl sulfoxide, dimethylformamide, and dimethylacetamide produced large and rapid changes. All of these compounds have aprotic molecules and form strong hydrogen bonds. They also have high lipid and water solubilities. Diethylformamide and tetramethylurea were without observable effect despite their strong solvent powers. Dimethyl sulfoxide–water mixtures containing less than 50% dimethyl sulfoxide were inactive, but more concentrated dimethyl sulfoxide mixtures produced impedance changes which increased with concentration. Accelerants will be treated further in the section on vehicles.

If a substance is much more soluble in the stratum corneum than in the vehicle in which it is dissolved, when presented to the stratum corneum the concentration in the first layers of the stratum corneum at equilibrium may be much higher than the concentration in the presenting solvent. The concentration in the lower layers of the stratum corneum remains near zero, since these layers are in contact with a fluid that is being continuously replaced or through which diffusion is relatively rapid. The flux, therefore, is more accurately related to the difference in concentration in the top and bottom layers of the stratum corneum than to the difference in concentration in the solutions on the two sides of the membrane (126). The concentration in the top layer of the stratum corneum is determined by the relative solubility of the penetrant in the stratum corneum and the vehicle, *i.e.*, the partition coefficient (K_m). Fick's law is now expanded to:

$$J_s = \frac{K_m D_m}{\delta} \Delta C_s \qquad \text{(Eq. 2)}$$

where δ is the thickness of the stratum corneum. (This term was treated previously.) It is now seen that:

$$k_p = \frac{K_m D_m}{\delta} \qquad \text{(Eq. 3)}$$

Thus, after k_p and K_m are determined experimentally and δ is known, D_m can be calculated. The nonhomogeneous nature of the stratum corneum and the complications and uncertainties attendant upon an accurate determination of K_m and δ would appear to pose serious limitations in developing a satisfactory diffusion constant (D) for human skin (126). Yet, the equations describe reasonably well the permeability of the skin to nonelectrolytes.

Blank and Scheuplein (31), in studies of homologous alcohols from methanol to octanol, noted that water solubility decreased and lipid solubility increased with increasing molecular weight and the rate of penetration increased as the molecular weight increased. The permeability constants were directly proportional to the partition coefficients. Thus, within a given molecular series [of constant or nearly constant diffusivity (D)], the observed steady-state flux

(J_s) and permeability constant (k_p) are directly proportional to the relative solubility of the substance in the skin, as expressed by the membrane–solvent partition coefficient (K_m) (124).

Solubility is certainly a very important factor in determining the rate at which a penetrant passes through a membrane. However, before solubility factors can be truly related to flux, the specific stratum corneum–vehicle partition coefficient rather than the ether–water partition coefficient must be known (31, 121). The positive correlations of the straight chain alcohols and of other compounds (25, 48, 80) with ether–water partition coefficients may be fortuitous. Lien and Tong (191), using computerized multiple-regression analysis, showed that, for many series of drugs, percutaneous absorption through intact skin is highly dependent upon the lipophilic character as measured by log partition coefficients. Electronic and steric terms appear to play minor roles.

The partition coefficient between the membrane itself and the solvent may be determined by allowing a weighed sample of stratum corneum to come to equilibrium with a small volume of a solution of a solute of known concentration. The loss in concentration is determined, and the concentration in the stratum corneum can be calculated from this loss (126).

Enough data are now available to indicate that not only the solubility of the penetrant in the stratum corneum but also the ease with which it diffuses through the stratum corneum influences the flux. As polar groups are added to the diffusing molecule, forces of attraction between these groups and polar sites within the stratum corneum increase and the diffusivity of the molecule therefore decreases (124, 126). The polarity is affected also by the pH. The best absorption takes place when the concentration of nonpolar molecules is greatest, thereby positively affecting the diffusion gradient of drug by mass action in a direct ratio.

When the penetrant passes through the stratum corneum, it encounters the much less compact tissues of the stratum germinativum and dermis. No satisfactory way has been found for isolating the stratum germinativum as an intact membrane for measurement of its permeability. The relatively few comparisons that have been made between the permeability of the stratum corneum and the entire epidermis indicate that the stratum germinativum offers very limited resistance to movement of molecules. The stratum corneum offered three orders of magnitude greater resistance to diffusion than did the dermis in the study of penetration of alcohols (10, 35).

Molecular Characteristics of Penetrant—While there is little doubt that factors such as hydration and solubility are uppermost, molecular features such as size and shape must play a part in penetration. Surprisingly little is known of the importance of the molecular characteristics of the applied agent. There are few published reports in which permeability coefficients are correlated with the size of penetrating molecules. Virtually nothing is known of the effect of molecular shape.

An inverse relationship appears to exist between absorption rate and molecular weight (4, 31, 62, 80, 91, 192). Small molecules penetrate more rapidly than large molecules, but within a narrow range of molecular size, there is little correlation between size and penetration rate. Diffusion constants through the hydrated stratum corneum for many low molecular weight compounds are approximately the same (124). The specific effect on the penetration rate of the size and shape of the penetrating molecules can be determined only if the effect of size and shape can be separated from the effect of solubility characteristics. The effect of polarity was discussed previously.

Molecular characteristics, yet unresolved, are involved in penetration. Feldmann and Maibach (73) determined the human urinary excretion of a series of topically applied molecules of relatively similar molecular weight (~200). There was a large difference in penetration of the compounds tested. The range for total absorption was greater than 250 times, while differences in the maximum absorption rate were greater than 1000-fold. Closely related compounds showed great differences in penetration. Benzoic acid was absorbed 200 times more than its glycine conjugate, hippuric acid. Nicotinic acid showed minimal penetration while 10% of its amide, nicotinamide, was absorbed. Compounds such as hippuric acid, nicotinic acid, and nitrobenzene support the generally held view of the effective barrier properties of the skin; others such as dinitrochlorobenzene, caffeine, and benzoic acid penetrate so extensively as to suggest that the human skin has little barrier properties to them.

Higher molecular weight materials also show variable penetration. Tregear (192) measured the rabbit and human skin penetration of large molecules such as serum albumin, polyvinylpyrrolidone, and two dextrans having molecular weights of 9400 and 153,000. The albumin penetrated the skin at a slower rate than it penetrated the excised skin. The labeled polyvinylpyrrolidone penetrated the slowest; the lower molecular weight dextran penetrated the fastest. Very large molecules such as proteins and polysaccharides went through very poorly, if at all. The use of dimethyl sulfoxide as a penetration carrier indicated that substances having molecular weights of 3000 or more cannot be transported into the skin (193).

Vehicles—The influence of formulation and selection of particular vehicles has been neglected from the point of view of their penetration of the skin. Few studies or techniques apparently can be used routinely to observe the role that a vehicle, or a particular component in a vehicle, will play on the overall percutaneous absorption of a drug. The lack of these studies can be partly related to the difficulties of routinely setting up percutaneous absorption studies and also to the emphasis given to other factors during the development of a topical vehicle or pharmaceutical product. The emphasis has generally been placed on the compatibility, stability, and appearance of the product rather than on the influence that the components in the vehicle may have on enhancing or hindering the movement of the drug through the skin

(123). It is only quite recently that experimental and clinical evidence has been produced to suggest that the vehicle can materially affect skin penetration (50–54, 84, 175, 184, 189, 194, 195). Particularly notable is the critical review of Poulsen (195) who analyzed the factors involved in optimizing drug availability from topical dosage forms.

The literature on the influence of vehicles on skin penetration is confusing and sometimes contradictory because: *(a)* a variety of experimental animals have been used, *(b)* many different methods of estimating skin penetration have been used, *(c)* there is a lack of awareness of possible drug–vehicle interactions and of the functions of different vehicles (10, 11, 196), and *(d)* there is a lack of consideration of the thermodynamics involved in the interpretation of results (10, 12, 121, 197).

Rothman (1) reviewed the literature on vehicles up to 1954, and Barr (7) reviewed this literature to 1962. More recent reviews have been prepared (2, 5, 8, 11, 50, 116, 196, 198, 199). The bulk of recent data on vehicular effects in percutaneous absorption has been derived from studies of corticosteroids (11, 17, 51, 63, 84, 116, 118, 119, 175, 194, 198, 200–205). Katz and Poulsen (17) discussed the interactions among the drug, the vehicle, and the skin. Their goal was the design of a dosage form that not only had good physical stability and patient acceptability but also that provided the optimum environment for the release of the corticoid from the vehicle and its penetration through the skin barrier.

The primary requirement for topical therapy is that a drug incorporated in a vehicle reach the skin surface at an adequate rate and in sufficient amounts. At one time, the primary factor influencing penetration through the skin was believed to be the vehicle itself. The bulk of evidence now indicates that unless an applied material is capable of passage through either the skin barrier or follicles, the vehicle is only of subsidiary importance. Hence, there are two general approaches to the problem of the development of vehicles that may increase penetration. One is to include agents in the vehicle that affect the barrier function of the epidermis so as to promote penetration of the therapeutic compound (111, 118, 119, 194). The other is to alter the physical characteristics of the vehicle and thus affect the diffusion of the drug from the vehicle into the skin (51, 54).

For the latter, it is advantageous to choose vehicles that do not bind the incorporated drug too strongly, because the drug has to separate from the base before it enters the cells. *In vivo* (52) and *in vitro* (31) studies have shown that the release of a substance will be favored by the selection of vehicles having a low affinity for the penetrant or in which the drug is least soluble. This finding is consistent with the view that the rate of release is governed by the vehicle to receptor phase (stratum corneum) partition coefficient.

For the first approach, penetration may be enhanced by a vehicle in which the drug is soluble if the solvent lowers the resistance of the skin to penetration either by producing changes in the transepidermal barrier or by modification of the follicular and sebaceous route, such as with dimethyl sulfoxide. Other drug–vehicle factors that are likely to be important are the particle size of poorly suspended drugs, the viscosity of the vehicle, and the drug concentration in the vehicle. A reduction in the particle size of fluocinolone acetonide has been shown to enhance its penetration (200, 201). Where a drug exists in more than one crystalline form, the one with the highest thermodynamic activity would be expected to penetrate most rapidly, provided it is stable (12, 121).

While vehicles appear to play a secondary role in transepidermal absorption, they are of considerable significance in enhancing absorption by the transappendageal route. Notable in this context is a vehicle consisting of water, propylene glycol, and wetting agents (44, 46). Increases in strictly perifollicular physiological responses to pilocarpine, histamine, and adrenalin were noted, with no significant changes in areas without follicles (44). While it is probable that wetting agents are the prime reason for the increase in transappendageal absorption, the physical nature of the base is also important.

The main factors in the physicochemical relationship of the penetrant to the vehicle appear to be the solubility of the penetrant in the vehicles or a constituent of the vehicle, the rate of diffusion of the penetrant within the vehicle, the rate of release of the penetrant from the vehicle, and the possible release of the penetrant in solubilized form together with a constituent of the vehicle (51, 195, 206). The one variable that can be altered is the thermodynamic activity of the drug in the vehicle. This thermodynamic activity is the product of the concentration of the drug and its activity coefficient in the vehicle. In a study of the effect of thermodynamic activity on the percutaneous absorption of methyl nicotinate from a water–glycerol mixture, the time of onset of erythema was the same for solutions of equal thermodynamic activity (207).

For most substances, the rate of penetration is limited by the impermeability of the skin. In such cases, the highest thermodynamic potential in the applied phase is necessary to obtain the maximum rate of penetration. Many investigators have indicated the importance of the drug concentration in a topical preparation, but few have indicated that the activity coefficient may be changed in many ways. Some investigators increased the concentration of the drug in the vehicle but actually lowered thermodynamic activity because they apparently were not aware of the effect of pH and other factors.

Higuchi (121) pointed out that the driving force behind the drug movement is the difference in the thermodynamic potential between the vehicle and the deeper tissues, and the direction of flow for systems is always from the higher thermodynamic potential to the lower thermodynamic potential. He derived the following equation describing the variables affecting the rate of release of solid drugs suspended in a vehicle:

$$\frac{dq}{dt} = \frac{1}{2}\sqrt{\frac{D(2A - C_s)C_s}{t}} \qquad \text{(Eq. 4)}$$

where:

dq/dt = rate of drug released per unit area of surface exposure
A = total concentration of the drug in the vehicle (suspended and dissolved drug)
C_s = solubility of the drug in the external phase of the vehicle
D = diffusion constant of the drug molecule in the external phase of the vehicle
t = time at which the amount of drug released is determined

When much more solid drug is present than is required to saturate the external phase of the vehicle ($A \gg C_s$), Eq. 4 can be simplified to:

$$\frac{dq}{dt} = \sqrt{\frac{ADC_s}{2t}} \qquad \text{(Eq. 5)}$$

Since the skin probably does not function as a perfect sink, absorption measurements would not be expected to agree initially with the rate of release and, consequently, the rate of absorption predicted by Eq. 5. However, once the rate of drug penetration across the major barrier reaches a steady state, absorption by the lower tissues should approximate the value predicted. Whether absorption by the epidermal and dermal phases would be proportional and whether absorption by either phase would agree with the absorption predicted by Eq. 5 would depend again on the relative affinity of the penetrating drug for each phase. There is evidence that absorption by the two phases of a drug suspended in systems such as described by Eq. 5 is not proportional (12, 121, 197, 208).

The permeability constant quantifies C_s and D as the product of the effective partition coefficient (PC) of the drug between the skin barrier and the vehicle and its diffusivity (D_b) in the skin or:

$$\text{permeability constant} = (PC)(D_b) \qquad \text{(Eq. 6)}$$

Using this equation, the rate of percutaneous absorption of drugs, applied as fine suspensions, can be described as a function of the product of the amount of drug released from the vehicle to the absorption site and its permeability constant for transport through the barrier phase. Combining the previous equations yields:

$$\frac{dQ}{dt} = \sqrt{\frac{ADC_s}{2t}}\,(PC)(D_b) \qquad \text{(Eq. 7)}$$

The release rate of a drug from the vehicle may be regulated by controlling A, D, and C_s. If an aqueous solution is the external phase of the vehicle, C_s can be varied by changing the effective pH of the vehicle for poorly soluble, weakly acidic and basic drugs. The activity coefficient of the molecular form of such drugs (the molecular or unionized form being assumed to be the species absorbed) is a rapidly changing function of pH for pH values greater than the pKa for acidic drugs and less than pKw − pKb for weakly basic drugs. The activity of the molecular species of a weakly acidic drug will be higher at low pH values and the activity of molecular species of a weakly basic drug will be higher at higher pH values (121). Thus, a weakly acidic compound, buffered to a weakly acid pH, will have a higher activity than if it were buffered at an alkaline pH and, consequently, its release will be dramatically improved. The converse is true of weakly basic compounds (11).

Table II—Permeability Constants (cm $hr^{-1} \times 10^3$) for Ethanol and Heptanol when Penetrating from Different Vehicles[a]

Alcohols	Vehicles		
	Water	Isopropyl Myristate	Mineral Oil
Ethanol	0.6	9.5	20.0
Heptanol	35.0	0.2	1.0

[a] From Ref. 35.

Equivalent relationships can be used to approximate the penetration of the barrier phase of the skin by a drug dissolved in a topical vehicle:

$$\frac{dQ}{dt} = (PC)C_v\frac{DA}{L} \qquad \text{(Eq. 8)}$$

$$\frac{dQ}{dt} = \frac{a_v DA}{\gamma_s L} \qquad \text{(Eq. 9)}$$

where:

dQ/dt = steady rate of penetration
(PC) = effective partition coefficient of the drug between the skin barrier and the vehicle
C_v = concentration of the drug in the vehicle
D = effective average diffusivity of the drug in the skin barrier
A = cross-sectional area of the application site
L = effective thickness of the skin barrier
a_v = thermodynamic activity of the drug in the vehicle
γs = effective activity coefficient of the drug in the skin barrier

If Eqs. 4 and 5 are considered with the objective of altering the penetration of a topically applied drug, it becomes apparent that only certain terms are readily susceptible to manipulation by the formulator. These terms are the partition coefficient (PC) and drug concentration (C_v) terms of Eq. 8 and, equivalently, the activity term (a_v) of Eq. 9 (51). Relationships between partition coefficients of various compounds and skin penetration have been reported (25, 80, 184, 209, 210). The partition coefficient can be defined as:

$$PC = \frac{\gamma_v}{\gamma_s} \qquad \text{(Eq. 10)}$$

where γ_v and γ_s are the activity coefficients of the drug in the vehicle and skin, respectively. While it may be necessary to regard the activity coefficient of the drug in the skin barrier phase as an unknown and unalterable value, γ_v can be modified simply by altering the composition of the vehicle. The mathematical analysis of permeability data has been reviewed (16, 206).

For a given concentration of drug in certain vehi-

cles, the activity coefficient of the drug and, consequently, the thermodynamic activity of the drug in the vehicle at that concentration may vary by a factor as much as a 1000-fold (121) from one vehicle to the next. Solutes held firmly by the vehicle, such as when the drug forms a soluble complex with the vehicle, exhibit low activity coefficients; hence, the release rate from such drug–vehicle combinations is slow. Solutes held "loosely" by the vehicle (less affinity of the vehicle for the drug or solute) exhibit high activity coefficients; therefore, the rate of release from such drug–vehicle combinations is faster (121).

Blank and Scheuplein (31) and Scheuplein (35) demonstrated the variability of permeability constants when alcohols, such as polar ethanol and nonpolar heptanol, are dissolved in solvents other than water (Table II). Ethanol penetrates better from the oils than from water, but the reverse is true for heptanol. Moreover, the penetration for both alcohols from the two oily vehicles varies. It is reasonable to assume for each of these alcohols that once the alcohol is transferred to the skin from any vehicle, the diffusivity through the stratum corneum (D_m) does not vary. Therefore, the change in flux for any alcohol from different vehicles must result from changes in the partition coefficient as the vehicle is varied. The polar alcohol tends to stay in the polar vehicle and not be transferred to the skin, but it is transferred from oily vehicles; the reverse is expected for the nonpolar alcohol.

Wagner (12) pointed out that thermodynamic considerations can help explain varied results with salicylic acid (165, 211–215) and sulfanilamide (211, 215) used as tracers in dermatological research. The activity coefficients of these tracers and their thermodynamic activities at a given concentration in the various vehicles would be expected to vary widely just on the basis of the nature of the vehicles and tracers employed in these studies. Polyethylene glycols complex salicylic acid; therefore, one would expect the thermodynamic activity of salicylic acid at a given concentration in polyethylene glycol ointment to be very low. Barrett *et al.* (196) noted that polyethylene glycol slowed down the penetration of methyl nicotinate and attributed this finding variously to an adverse diffusion coefficient in the vehicle, to an inability to hydrate the stratum corneum, or to an osmotic effect which would tend to dehydrate the horny layer. Commercially available formulations of corticoids were compared by the vasoconstriction test (216). Ointment vehicles were generally superior to creams or lotions.

The literature with regard to the advantage of one type of base over another is conflicting, yet the efficiency of various vehicles in aiding penetration can be reasonably predicted on the basis of their effect on *(a)* hydration of the stratum corneum or *(b)* the activity of water in the stratum corneum which influences the stratum corneum–vehicle partition coefficient.

Greases and oils are the most occlusive vehicles and induce the greatest hydration through sweat accumulation at the skin–vehicle interface (171). This process is accentuated by covering the surface with occlusive bandages or plastic. Emulsions of the water-in-oil type are somewhat less occlusive than greases. Substances in the vehicle that have a high affinity for water, such as humectants, tend to dehydrate the stratum corneum and decrease penetration. Similarly, powders increase surface area, increase the rate of evaporation of water, and decrease the extent of hydration (171).

The evidence of the role of a solvent, such as propylene glycol, in skin penetration is conflicting (46, 50, 51, 74, 175, 194, 201–205, 217). In combination with surfactants, propylene glycol was used to promote the penetration of water-soluble substances *via* the transfollicular route (46). It has been used as the preferred vehicle for the topical application of fluorouracil in the treatment of certain types of skin cancer. A concentration of 1% fluorouracil has been found to be as effective clinically as a minimum concentration of 5% in an ointment (217). Enhanced effects were noted in absorption of fluocinolone acetonide (201, 202, 205), betamethasone valerate (50), fluorometholone (204), flucloronide (175), and beclomethasone dipropionate (204). However, Feldmann and Maibach (74) failed to confirm these findings in a study of the percutaneous penetration of hydrocortisone.

Ostrenga *et al.* (206) showed that, at moderate concentrations, propylene glycol has no apparent effect on the permeability of the skin; but above 50%, the results suggest that the permeability of the barrier is decreased. The varying effect of propylene glycol on the penetration of different drugs is probably due to their different solubilities. Penetration is probably greatest when the drug is present at its maximal solubility concentration. Below this level, the affinity for the drug may be greater than that of the skin and penetration is prevented (199). Dimethyl sulfoxide and dimethylformamide increased the penetration of hydrocortisone three to four times, whereas dimethylacetamide and propylene glycol decreased it. These workers used undiluted solvents in their investigations, which may account for the apparent anomaly between their results and those of others (119, 194, 201, 202).

In a comparison of solvents, Brode (205) showed that, using ethanol as a standard, absorption of fluocinolone through rat skin was 9 for dimethyl sulfoxide, 5 for propylene glycol, and 2 for tetrahydrofurfuryl alcohol. Absorption from a polyethylene glycol base was less than 20% of that from ethanol solution; from both aqueous and oily creams, the absorption was 80%; and from white soft paraffin, it was 60%. These results are in agreement with those obtained in human subjects (194, 201).

Dempski *et al.* (189) noted that dexamethasone was released more rapidly from gelled isopropyl myristate than from petrolatum and two other nonaqueous vehicles. Their studies demonstrated that the *in vitro* release of medicinal agents from topical bases is a function of the solubility of that agent in both the base and its surrounding medium. The medicinal must be sufficiently soluble in a nonaqueous base to

allow for its release into an aqueous medium but not so soluble to remain in that base preferentially. If the drug is insoluble in its vehicle, only the drug particles available at the surface of the vehicle apparently dissolve into an aqueous medium. If the drug is partly soluble in the vehicle, it seems to dissolve and diffuse throughout the medium as it dissolves from the surface and then returns to the surface for release into the surrounding medium.

The vasoconstrictor activity of betamethasone 17-benzoate was studied in several ointment bases (218). Preparations containing the steroid in petrolatum–propylene glycol or in petrolatum–isopropyl myristate both gave rise to significantly higher total vasoconstriction than polyethylene glycol ointment BPC, the latter with varied amounts of propylene glycol, Plastibase, and a petrolatum–beeswax base (218).

The role of emulsions, particularly with regard to oil-in-water and water-in-oil-bases, is unresolved (see Refs. 1 and 7 for reviews through 1962). Salicylic acid has been studied frequently in various vehicles (165, 219–224), but the role of the vehicle is not yet understood. Barrett *et al.* (196) showed that methyl nicotinate penetrated the skin equally readily from an aqueous or oily cream. Using the steroid fluocinolone acetonide, Munro (116) demonstrated that a petrolatum ointment gave the best penetration, followed by a lotion and cream base and then by propylene glycol. Pure propylene glycol apparently inhibits penetration. This report confirmed the findings of Poulsen *et al.* (51) who indicated that maximum release of fluocinolone acetonide was obtained from vehicles containing the minimum amount of propylene glycol required to dissolve the steroid. Increasing the concentration of an effective solvent may, in fact, result in reduction of the amount released.

With betamethasone valerate, no marked difference in penetration was observed from an oil-in-water cream, water-in-oil cream, petrolatum ointment, or polyethylene glycol ointment (194). Glycols, such as propylene glycol and 1,3-butanediol, enhanced the action of ointments containing the same steroid (50). The potentiating action of glycols may be due to the droplets acting as an efficient reservoir of dissolved corticosteroid readily available to the oily phase. A mechanism whereby propylene glycol penetrates the skin and carries corticosteroid with it might also be involved. The results obtained with the steroids must be regarded with caution, since virtually all techniques measure the blanching of the skin produced by vasoconstriction. Assessment of the pallor can be influenced by the refraction and reflection of light by the vehicles.

The penetration of topical steroids through human abdominal skin was studied (225). Release, penetration, and *in vivo* data were compared as a function of vehicle composition. The similarity between the *in vivo* and *in vitro* composition profiles suggested that clinical efficacy can be predicted from *in vitro* data and from the physical properties of the steroids. The correlations indicated that the *in vivo* results were directly dependent upon penetrability.

The effect of vehicle composition on steroid release from ointments was studied (51, 88, 206, 226). Inferior release of fluocinonide was obtained in conventional ointments in which the drug was present as micronized particles. Greatly improved bioavailability was obtained from an ointment delivery system in which the steroid was completely dissolved. The ointment system consisted of a propylene glycol solution of the drug dispersed in petrolatum, which delivered the corticosteroid to the skin in a molecular form but retained the classical function of the ointment as an occlusive, protective covering. Similar improvements were noted with the use of an anhydrous fatty alcohol–propylene glycol cream base over a traditional two-phase emulsified cream (227). Time–response curves were constructed (88). These fatty alcohol–propylene glycol vehicles have the physical properties of gels but resemble creams in appearance and consistency.

All cited steroid studies dealt with the use of a skin membrane either *in vitro* or *in vivo*. Recently, solvents such as alcohol–water have been utilized as a "model" to simulate the skin phase (50–54, 207, 225). The vehicle has negligible solubility in the phase representing the skin, but the steroid is fairly soluble in it. A receptor phase or "sink" is used to receive the penetrant. Chloroform (50, 52) and isopropyl myristate (50, 51) have served as sinks. Since they are immiscible with the alcohol–water solvent, it is not necessary to introduce an artificial membrane to separate it from the vehicles.

Poulsen *et al.* (51) measured the release of fluocinolone acetonide and its acetate ester into an isopropyl myristate phase. The vehicles used were mixtures of propylene glycol and water gelled with carbomer[2] and diisopropanolamine. The studies showed that optimal release was obtained from vehicles containing the minimum concentration of propylene glycol required for complete solubilization of the corticosteroid. The poorest release rates were obtained with very high concentrations of propylene glycol. The results indicated that the important factors influencing the release of steroid into the receptor phase were the solubility in the vehicle and the partition coefficient of the steroid between the vehicle and the receptor phase. The findings appear to indicate that each compound requires individual formulation based on its solubility characteristics and that the formulation may also need modification for different concentrations of the agent to obtain maximal release rates (53, 206, 225). Similar studies using isopropyl myristate found striking correlations between *in vitro* release rates and *in vivo* results (206, 225).

Busse *et al.* (50) used both chloroform and isopropyl myristate as separate receptor phases in studies of betamethasone valerate in ointment bases. The rate of release of the steroid into the chloroform phase from a paraffin ointment was about 4.5 times that from a similar ointment containing 10% hydrogenated lanolin. Conversely, in the isopropyl myristate system, the reverse occurred. The *in vitro* results

[2] Carbopol 934.

suggest that the isopropyl myristate system more adequately represents the skin.

The data of the model systems can be considered in terms of the rate of solution of corticosteroid particles in the base and the rate of transfer of corticosteroid from the base (50). The latter rate is related to the partition coefficient between the base and the skin or phase representing the skin. When the partition coefficient is large due to the low solubility of the corticosteroid in the base, there is a greater tendency for transfer of corticosteroid to the skin. A second factor is related to the concentration of steroid dissolved in the base (50). Blank and Scheuplein (31) considered that differences in penetration from vehicles can be explained by differences in the stratum corneum–vehicle partition coefficient without assuming that one vehicle penetrates more readily than another. From work using receptor phases, it should be possible to predict *in vivo* effects with suitable models (50, 53, 206, 225).

Vehicles may also affect penetration by their ability to reduce water vapor loss on the skin surface. Experimentally, paraffin bases have been shown to suppress transepidermal water diffusion, whereas a number of other standard vehicles cause a lesser degree of transepidermal water loss suppression (228). Surprisingly, anhydrous wool fat failed to suppress water loss in this study. It was also postulated that the increased penetration of corticosteroids from vehicles containing solvents, such as tetrahydrofurfuryl alcohol and propylene glycol, was due to the influence of the latter on the barrier function of the horny layer. The evidence for this assumption is based on the increased water loss through the horny layer brought about by vehicles containing these solvents. Therefore, it appears that two contradictory mechanisms may be involved in vehicle effects. The occlusive nature of the vehicles is credited with retention of water and subsequent improved penetration, but an increase of water loss may be associated with reduced barrier function and, consequently, easier passage of the corticosteroid (198).

Lipophilic solvents have been reported to facilitate penetration. The absorption of water-soluble and lipid-soluble substances from terpenes and terpene derivatives was better from eucalyptol than from alcoholic solutions (229). A vehicle containing *p*-cymene was superior to others tested in promoting the penetration of hormones but had some irritating effects (230). Small differences were found in the penetration of physostigmine from alcoholic and ester solutions at a rate dependent on the aliphatic chain length (231).

SORPTION PROMOTERS

In the past 10 years, attention has focused on methods of increasing the rate of absorption of topically applied drugs. So far, the one method that has come into everyday use is the application of topical corticosteroids under thin plastic film (168, 169, 232, 233). This method now has widespread application in the treatment of recalcitrant psoriasis. Another method is to add materials that can combine with, or dissolve in, the structures of substances making up the barrier. These agents have come to be known as accelerants. To increase permeability, the accelerant causes the keratin to swell and leaches out essential structural material from the stratum corneum, thus reducing the diffusional resistance and increasing the permeability (36, 101, 182, 234, 235).

Varied agents have been reported as sorption promoters, such as the hydrophilic solvent propylene glycol or surfactants. However, the most effective accelerants are aprotic materials such as urea (236), dimethyl sulfoxide, dimethylformamide, and dimethylacetamide. Work with dimethyl sulfoxide and other accelerants is contributing to the understanding of the chemical nature of the skin barrier in relation to a specified penetrant and the transport mechanisms of various compounds across skin.

Dimethyl Sulfoxide—On application to the skin, the dipolar aprotic solvent dimethyl sulfoxide passes rapidly through the stratum corneum (20, 147, 237–239) and can aid in the penetration of numerous substances. This process does not change the structure of the skin. The role of dimethyl sulfoxide in enhancing percutaneous migration is well documented (6, 20, 77, 101, 118, 148, 240–242). A series of patents was issued for the topical use of dimethyl sulfoxide to aid tissue penetration (243).

In vivo, dimethyl sulfoxide has been found to accelerate the penetration through the skin of water (146, 241), dyestuffs (237), hexopyrronium bromide, naphazoline, fluocinolone acetonide (118), salicylic acid (244), tubocurarine hydrochloride, amphetamine sulfate, barbiturates (245), and other substances (93). *In vitro,* dimethyl sulfoxide accelerates the penetration of skin by hexopyrronium chloride and hydrocortisone (118), picric acid (182, 234, 235), fluocinolone acetonide, triamcinolone acetonide, hydrocortisone (20), griseofulvin and hydrocortisone (119), testosterone propionate (70, 77), hydrocortisone (77, 111, 113), triamcinolone acetonide (113), fluocinolone acetonide (111, 246), cortisone acetate and estradiol (247), fluorouracil (248), iodine (249), hexachlorophene (250), physostigmine and phenylbutazone (251), local anesthetics (252), antibiotics (244, 253), and quaternary ammonium compounds (254).

Substances having molecular weights of 3000 or more cannot be transported into the stratum corneum (255). The influence of pH on the percutaneous absorption of salicylic acid, with and without added dimethyl sulfoxide, from a hydrophilic ointment was studied in the rabbit (256). With those ointments containing dimethyl sulfoxide, salicylate levels were higher at each pH and time interval. Enhanced solubility of salicylic acid in the presence of dimethyl sulfoxide would appear to explain the results.

Dimethyl sulfoxide exerts variable influence even over structurally related compounds. The rate of migration of potassium methyl sulfate increases with an increasing concentration of dimethyl sulfoxide (183). Yet applications of the butyl and lauryl esters produces low penetration, independent of dimethyl sulfoxide concentration. In these latter cases, dimethyl

sulfoxide functions as a penetrant rather than as a vehicle (183, 257).

Dimethyl sulfoxide exhibits an unusual concentration dependence (146, 147, 182, 183, 257). The penetration rate of pure dimethyl sulfoxide depends on the concentration applied. At least 60% dimethyl sulfoxide is required for a measurable penetration rate. The rate rises to a maximum at about 3 hr and then declines; this is true for both the pure liquid and for dimethyl sulfoxide–water mixtures containing >70% dimethyl sulfoxide. The continued increase in the penetration rate of pure dimethyl sulfoxide with an increase in the applied quantity is probably a consequence of progressive barrier impairment. The decline in the peak rate after 3 hr is probably due to the back-diffusion of water with consequent dilution of the dimethyl sulfoxide (147).

Dimethyl sulfoxide can also establish a reservoir in the stratum corneum of compounds having a low water solubility and to which the epidermis is normally only slightly permeable (119). The related compounds dimethylacetamide and dimethylformamide also enhance cutaneous penetration (119) and retain agents in the stratum corneum reservoir (111, 258) but to a lesser degree than dimethyl sulfoxide (119).

Little information is available concerning the mechanism of action by which dimethyl sulfoxide, dimethylformamide, and dimethylacetamide enhance the rate of migration. They are all strongly hygroscopic, and it is likely that the presence of these substances in the stratum corneum greatly increases the hydration of the tissue and, therefore, its permeability. Two factors, the underlying dermal inflammation and the heat produced by the interaction of the solvent and water within the stratum corneum, would also lead to a small rise in the temperature and, therefore, the permeability of the horny layer (259). Neither of these factors could conceivably explain the magnitude of the increased diffusion. There is ample evidence of the ability of water (91, 146), and many other substances to move in an inward direction through the skin under the influence of dimethyl sulfoxide (51, 242). The accelerant effect observed *in vivo* is not due to increased skin circulation because this can be increased without increasing the penetration rate (91), because accelerants do not increase the skin clearance rate (101), and because the effect can be observed *in vitro* with isolated nonperfused skin preparations.

On a structural level, the possible reversible configurational changes in protein structure brought about by substitution of integral water molecules by dimethyl sulfoxide is worthy of special consideration (260). Also, dimethyl sulfoxide may function as a swelling agent (235). A combination of the latter two factors may explain in part the variable role of dimethyl sulfoxide since the swelling phenomenon may induce the formation of channels within the matrix of the stratum corneum, which favor the passage of various compounds (183), or lower the diffusional resistance of the stratum corneum. Dimethyl sulfoxide is able to extract soluble components from the stratum corneum, suggesting the possibility of ultrastructural modifications consistent with an increase in permeability (261). For a liquid to be a good accelerant, it must also release the penetrant readily to the aqueous milieu of the viable epidermis. This process could be hindered by an excessively unfavorable partition coefficient or by the extremely low water solubility of a penetrant (101). A possible explanation is that the flux of water into the dimethyl sulfoxide solution increases the thermodynamic activity of the drug and, hence, the penetration rate of drug into and through the skin (246).

Although the discovery of the rapid penetration through the skin of dimethyl sulfoxide suggested a major advance in therapeutics, the initial enthusiasm for this and similar solvents has not continued, partly because of their irritant action, odor, and toxicity.

Surfactants—Surfactants are often included in pharmaceutical formulations as emulsifying agents, but they probably have little effect on skin penetration. Yet, when the epidermis is treated with compounds having significant surface activity, its permeability to water alters (62, 262). One effect of surfactant action upon the skin may be to change the physical state of water in the skin in such a way as to permit greater freedom to the passage of charged, hydrophilic substances (62).

Several studies reported the effect of soap and surfactants on the loss of water (41, 149–152, 263–265). The role of surfactants in percutaneous absorption has been reviewed (7, 62, 71, 160, 240). Possible mechanisms whereby the skin is able to restrict the percutaneous migration of synthetic anionic surfactants have been well reviewed (145), and it was made clear that the factors involved may be of a complex nature. The irritant action of anionic materials, *e.g.*, "soap" or sodium lauryl sulfate, suggests that they must penetrate to susceptible tissue. It seems reasonable to assume that if a potential irritant is placed on the surface of the skin, penetration as far as the Malpighian layer of living cells is necessary for it to exert its irritant effect (149).

In the case of soap and other surfactants, the bulk of evidence indicates that the stratum corneum is an effective barrier (71, 102, 108, 127, 145, 183, 257, 266). While penetration is poor, there is evidence that anionic and cationic agents do penetrate the skin (71, 108, 127, 145, 150, 266), with anionics penetrating best followed by cationics and nonionics (134, 149).

Among anionic substances, the laurate ion is reported to have the greatest penetration and the greatest effect on the penetration of other solutes (150, 151). Soaps of different fatty acids have this property in varying degrees (149, 150). There is significant penetration by sodium salts of fatty acids with a chain length of 10 carbon atoms or less, and there is modest penetration by fatty acids with longer chains (62, 127, 145).

Previous investigations (127, 145) showed that none of a series of alkyl sulfates in the C_8–C_{18} range was able to penetrate whole skin samples until the samples had been pretreated with lipid solvents or the stratum corneum was mechanically removed.

This finding was corroborated for potassium lauryl sulfate (257). However, the lower molecular weight potassium methyl sulfate and potassium butyl sulfate are able to migrate across intact whole skin samples, with the methyl ester exhibiting the higher order of migration (183). Application of the lauryl ester and the butyl ester in dimethyl sulfoxide solutions did not alter the pattern of migration whereas the methyl ester showed a concentration-dependent accelerant relationship with increasing concentrations of dimethyl sulfoxide.

Sprott (62) found that alkyl sulfates did penetrate the skin of the rat, as did sulfonated fatty acids and carboxylic acid esters of isethionic acid. Measurements of the distribution of *n*-hexadecyl sulfate in the skin showed a very high concentration of this surfactant in the epidermis, suggesting that such anionic surfactants begin to penetrate into the dermis only after saturation of the epidermis. Hence, the inability of certain alkyl sulfates to migrate across whole skin samples is not a function of the whole class. The reduction of the alkyl chain length of these compounds leads to an increase in their rate of percutaneous migration. The sum effect of reduction in chain length is to increase the hydrophilic properties of the molecule and concomitantly reduce the lipophilic function. Anionic surfactants apparently bind strongly with skin protein and cause a reversible denaturation and uncoiling of the filaments, leading to a gross expansion of the tissue (267).

The penetration of fatty acid soaps has been shown to vary with pH (62, 127, 145). The penetration of fatty acid soaps varies inversely from pH 6 to 10.8. At higher pH, the action of the anionic surfactant upon the skin appears to be attenuated or overshadowed by the influence of the more alkaline pH itself. This result also suggests that any interpretation of surfactant action upon the skin must deal separately with these two phenomena, *i.e.*, pH and surfactant effect (62). However, Bettley (149), in a study of the penetration of varied surfactants, could find no correlation with pH.

Scala *et al.* (71) calculated permeability constants for a wide variety of materials including surfactants. The nonlinearity of diffusion curves (permeability constants *versus* time) for an anionic surfactant (sodium tetrapropylenebenzenesulfonate), a cationic surfactant (dodecyltrimethylammonium chloride), and soap probably means that the barrier to these surfactants is being altered by the surfactants themselves as they diffuse into and through the skin. A decrease in the barrier properties allows an increased diffusion, which results in a greater alteration of the barrier. Removal of the surfactant causes the process to stop at the stage of barrier alteration to which it has progressed.

Penetration of certain antimicrobial substances appears enhanced by addition of surfactants. Washing with sodium lauryl sulfate enhances the amount of hexachlorophene and tetrachlorosalicylanilide penetrating rat skin (62). The ability of anionic surfactants to promote absorption is confirmed by the observation that nickel salts compounded with anionic substances cause eczema, but nickel salts combined with nonionic or cationic substances do not. This difference was thought to be due possibly to an acanthotic effect or to a denaturing action on the epidermal protein by the anionic surfactants, thus resulting in the easier passage of the metal sensitizer through the skin (268).

The effects of the anionic surfactants on the penetration of water-soluble substances in the skin appear related to their ability to increase the permeability of the skin to water. Sprott (62) explained this effect in terms of a protein–surfactant interaction and its attendant effects. Thus, where surfactants attached to the skin proteins have brought about conformational changes upon the protein structure, the hydrophilic polar groups would have been forced into the interior of the protein helixes. Under those conditions, water and substances in aqueous solution, *e.g.*, iodide ions, would permeate the skin with enhanced facility since the individual water molecules in the vicinity of proteins so affected would be exposed to fewer electrovalently, *i.e.*, charged, polar groups.

Nonionic surfactants generally appear to have little effect in promoting skin penetration, although numerous reports on percutaneous absorption under the influence of nonionic substances have been published (212, 269–271). Penetration of hydrocortisone was enhanced by increasing concentrations of lauric diethanolamide, a nonionic surfactant, to a maximum at 7% and then was reduced, probably due to micellar trapping of the steroid (272).

The factor determining whether a nonionic surfactant will increase the absorption rate appears to be the configuration of the surfactant molecule rather than the hydrophilic–lipophilic balance or surface activity (273). Where the surfactant has several long hydrophilic chains (*i.e.*, more than five ethylene oxide units) rather than a single or several short ethylene oxide chains, drug absorption is not increased. This finding indicates that the effectiveness of the surfactant is due to the ease with which the surfactant molecules penetrate lipid membranes. Dugard and Scheuplein (274) also could not find a correlation between the hydrophilic–lipophilic balance and surfactant action, and they concluded that there was an action upon protein rather than lipid components of the stratum corneum.

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ACKNOWLEDGMENTS AND ADDRESSES

Received from the *Pharmaceutical Research and Development Department, Hoffmann-La Roche Inc., Nutley, NJ 07110*