JOURNAL OF Pharmaceutical Sciences

May 1962 volume 51, number 5

Review Article

Percutaneous Absorption

By MARTIN BARR

THE STUDY of the passage of medicinal substances through the skin offers a great challenge to research workers in the fields of pharmacy and dermatology. Although dermatological vehicles themselves may not penetrate the skin to any extent nor actually carry the medicament through the epidermal barrier, it is known that the clinical effectiveness of a drug may vary when it is incorporated in different vehicles (1). The choice of an optimum vehicle for a particular medicament depends on the physical and chemical properties of the drug alone and in the vehicle as well as the nature of the skin condition being treated. Much information has been gained on this subject in the last few years but much more is needed in order to treat the various skin disorders encountered with maximum effectiveness.

Intelligent formulation of dermatological preparations depends on a thorough understanding of percutaneous absorption. Although recent studies have led to a better understanding of this phenomenon, there are many uncovered facets concerning its mechanism. This paper serves as a review of studies made in the field of percutaneous absorption. Space limitations make it impossible to summarize every work in the field but an attempt has been made to review those which appear to be most significant to its understanding.

Before going further, it is appropriate to define the term "percutaneous absorption." Rothman (2) defines percutaneous absorption as the penetration of substances from the outside into the skin and through the skin into the blood stream. As the literature in this field is studied, it is found that many terms have been employed: namely, absorption, sorption, persorption, permeation, and penetration. As Blank (3) has stated, it is actually not necessary to define the term "percutaneous absorption" with great accuracy but it is of greater importance to know how molecules move into the skin from a unit area of cutaneous surface in a unit of time, and where these molecules go after leaving the skin surface.

MECHANISM OF PERCUTANEOUS ABSORPTION

The modern concepts of percutaneous absorption are relatively new, especially when compared to the long history of preparations used for application to the skin. The earliest records of the use of fats and oils as vehicles for cosmetics and medicaments date back to the early records of Babylonian and Egyptian medicine (4). Broadly sketching the historical views and experimentations, one finds that the subject may be divided into three periods: (*a*) the period antedating 1877 and covering 100 years, during which much of the evidence was accepted as indicating that a large number of substances, chiefly gases and volatile substances, penetrated the skin freely; (*b*) the period extending from 1877 until 1900, during which Fleischer's school of thought held forth, in which Fleischer and his collaborators concluded from their experiments, and a critical appraisal of the older observations, that the skin of man and higher animals was absolutely impermeable to all substances (5); and (*c*) the period from 1900 to the present, the first few years of which saw the decline of the absolute impermeability theory, chiefly under the impetus of Schwenkenbecher's work (6).

Received from the Department of Pharmaceutics, College of Pharmacy, Wayne State University, Detroit, Mich.

Despite the voluminous literature in the field, there is as yet very little agreement on the basic mechanism which is largely responsible for percutaneous absorption through intact skin. One group of investigators is of the opinion that the major role of entry for medicaments is directly through the intact epidermis (transepidermal route). Another group believes that most absorption takes place through the appendages, either through the pilosebaceous apparatus or the spiral sweat glands. There is evidence that indicates it is likely that all these avenues take part in the transfer of substances through the skin (7). No attempt will be made in this review to resolve this controversy. It is very likely that a specific substance may prefer one avenue to the other, depending on the many factors which will be discussed later in this paper.

Before reviewing the work that has been done on the mechanism of percutaneous absorption, it is appropriate to consider briefly the anatomical zones a penetrating substance meets in transit from the skin surface to the blood vessels (Fig. 1). In order, it encounters a surface film of emulsified lipids, the stratum corneum (horny layer), the barrier, the stratum germinativum (living epidermis), and finally the dermis (true skin) and blood vessels. Other avenues of penetration through the skin are shown diagrammatically in Fig. 2.

Transepidermal Absorption.—Mali (8) states that the pathway through the epidermis is much more likely the main avenue of penetration for substances than are the sebaceous glands or sweat glands, simply because the epidermis presents a surface area 100 or 1,000 times greater than the other two.

Various anatomical zones of the skin behave differently with regard to transepidermal absorption (9). The film on the surface of the skin is composed of sebum, sweat, and desquamating horny layer, and has a complex chemical composition. The character and extent of its interaction with the penetrating agent are unpredictable. This surface film is discontinuous and offers relatively little resistance to a penetrating molecule (9).

The horny layer, which is 20 to 40 μ in thickness, is composed largely of keratin, a sulfhydryl containing protein which adsorbs a large amount of water and other polar compounds. It also contains surface lipids which may spread along the channel walls between cells and adsorb lipid-soluble material. In other words, the horny layer may act as a sponge, becoming a reservoir for the penetrating agent and maintaining a maximum concentration gradient just above the barrier, thus possibly hindering penetration. This is especially true for ions and dyes of low molecular weight. Blank and Gould (10), using an autoradiographic technique, have shown that ionic surfactants tagged with S^{35} are bound by the horny layer and often do not penetrate beyond the orifice of the hair follicle (9).

The barrier zone was originally demonstrated by Rein (11) who reported that it was located between the horny layer and granular layer underlying it and that it was an electronegatively charged barrier which repelled anions and attracted and held cations for further penetration. Rothman (12) reported that the barrier layer checked the transfer of water across the skin. Blank (13) showed graphically that the barrier to water transfer in skin is a layer a few microns thick at the base of the stratum corneum. He used the plastic tape stripping method of Wolf (14) and Pinkus (15) to remove the horny layer gradually. He found that the diffusion of water through skin remained low until the base of the horny layer had been stripped away; thereafter the permeability to water rose sharply. In 1951 Szakall (16) reported that after the horny layer had been stripped off with tape, the entire barrier could be removed on Tesa tape in a single stripping. Subsequently he recovered the barrier layer by dissolving the tape in petroleum ether. Mali (8) isolated the barrier utilizing another method in 1955.

The barrier layer is 10 μ thick and prevents the penetration of molecules having molecular weights greater than 200 or 300 (17); yet the diameter of the pores in the material in the intercellular spaces in the barrier is much larger than the largest penetrating molecule. Thus, the restraining force must be the molecular interaction between penetrant and pore contents. If the substance has a high electrostatic charge, e.g., ions, the attraction is so great that no penetration occurs. If the substance has a water-lipid partition coefficient of about 1.0, it has the highest permeability; the barrier must then have both polar and nonpolar groups in the pore contents (9).

The few molecules which do penetrate the barrier layer are either bound by the living epidermis or the dermis, or carried away by the tissue fluids to the lymphatic and blood vessels. The electron-dense zone which is present at the junction between the epidermis and the dermis offers little resistance to the penetrating molecules (9).

Blank, Griesemer, and Gould (18) have pub-

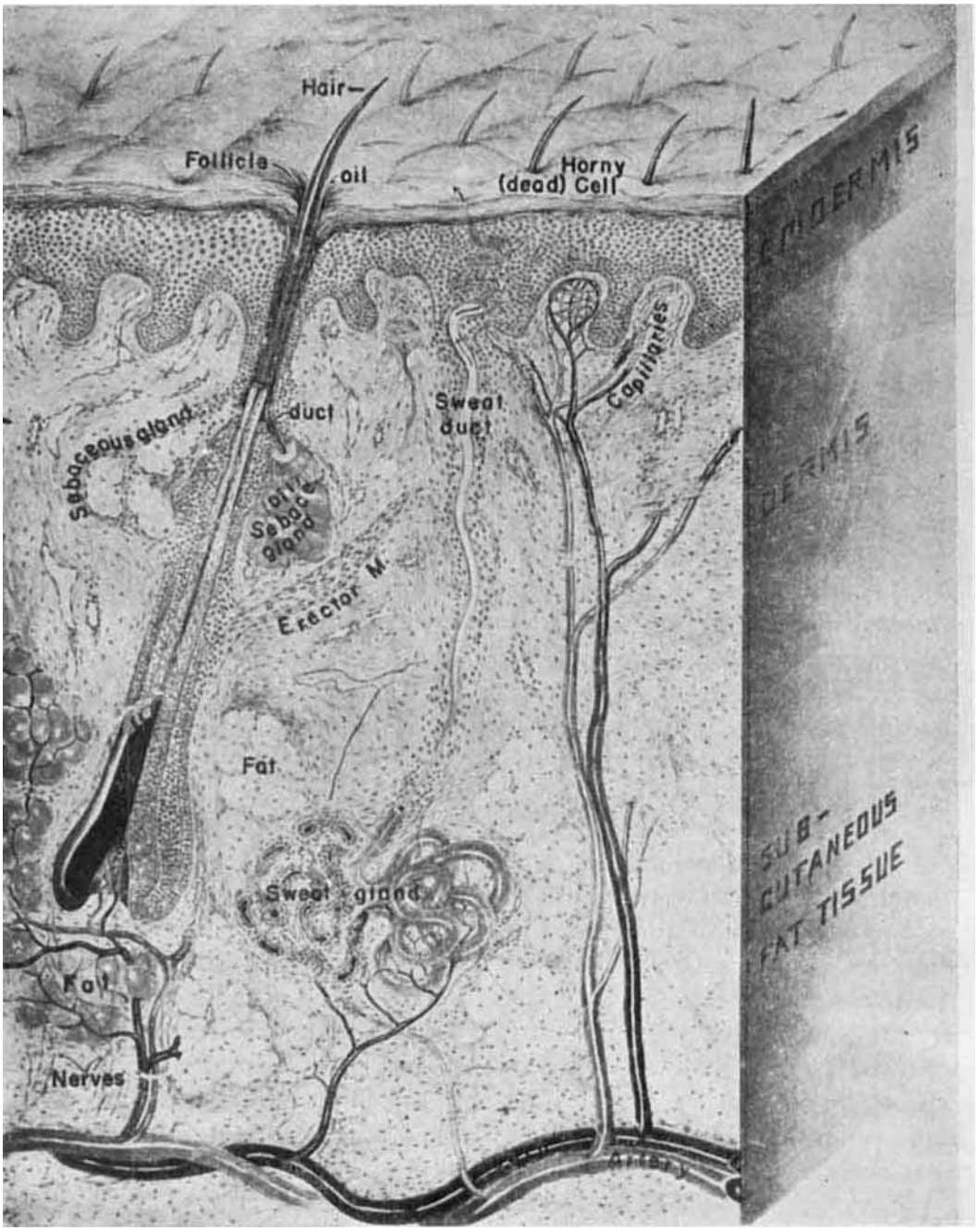

Fig. 1.—Anatomical zones encountered by a penetrating substance in transit from the skin surface to the blood vessels [from Griesemer, R. D., *J. Soc. Cosmetic Chemists*, **11**, 80(1960)].

lished autoradiograms of rabbit skin which had been exposed to the anticholinesterase agent, sarin, tagged with P^{32}. These indicate that penetration occurred through the transepidermal route. A similar route of penetration was described for hydrocortisone by Scott and Kalz (19).

It appears to be true that the ratio of the solubilities in water and in lipids, as originally advocated by Meyer and Overton, is actually important for the absorption of substances through the skin. Rothman (12) has shown that lipid-soluble substances penetrate the skin more readily than polar substances, and that substances soluble in both lipids and water penetrate most rapidly of all. Treherne (17) obtained quantitative data on the permeability of excised human skin to a series of radiotagged substances. He showed

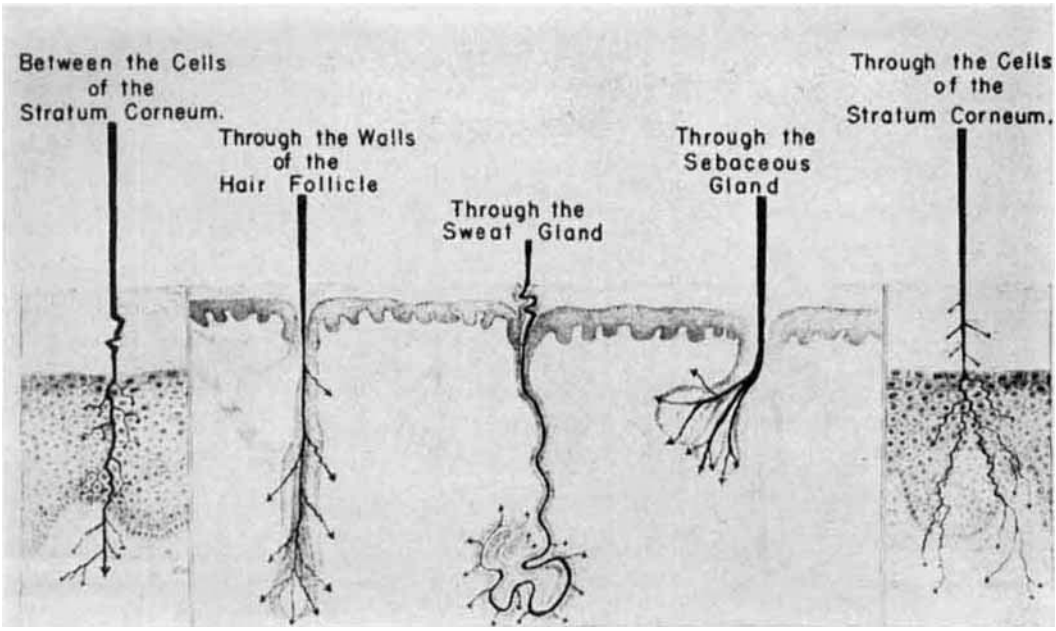

Fig. 2.—Possible avenues of penetration into and through the unbroken skin [from Griesemer, R. D., *J. Soc. Cosmetic Chemists*, 11, 81(1960)].

conclusively that the permeability of skin is directly proportional to the ether/water partition coefficients of these substances.

Absorption Through Appendages.—Hair follicles and sweat gland ducts open on the surface of the skin in the form of visible pores and these are believed to be avenues for the passage of medicaments through the skin. As has been previously stated, a majority of investigators believe that the hair follicles are the major avenue for percutaneous absorption.

Pilosebaceous Apparatus.—There are many studies which have shown the prominent role of the pilosebaceous apparatus in percutaneous absorption. This concept takes into consideration the solubility of the drug in sebum.

In the upper portion of the follicular canal, the hair shaft does not adhere to the follicular wall, and therefore a space is formed which is filled with horny scale and air. This interspace is continuous with the duct of the sebaceous gland. The sebum from this duct eventually empties into the interspace (20). Therefore, any medicament possessing a solubility in sebum may penetrate this space and reach inside of the sebaceous gland whose membrane is more permeable than the epidermal barrier. Similarly, the wall of the follicular sheath is less resistant to penetration than the surface epidermis. From the sebaceous glands and hair follicles, medicaments may penetrate downward into the corium and from there into the blood, thus bypassing the barrier. Medicaments may also move upward from the sebaceous glands into the epidermis but without penetrating the barrier layer. This pathway of absorption was demonstrated by MacKee, *et al.* (21), and by other workers (22, 23) who detected the presence of various tracers in the deeper parts of the hair follicles and in the sebaceous glands.

Sweat Ducts.—There is much controversy as to whether the sweat ducts serve as an avenue for percutaneous absorption. Rein (24) reported that he observed the staining of the pores of sweat ducts, like those of follicles, when he introduced dyes into the human skin by electrophoresis. Ichihashi (25) postulated that this "pore pattern" indicated absorption of the dye through the sweat ducts. Abramson, *et al.* (26–28), agreed with Ichihashi's conclusion and found the development of the sweat pore patterns dependent on the electric charge of the skin. On the other hand, Flesch (29) could not demonstrate penetration of dye into sweat ducts. Other studies (30–32) militate against any substantial penetration or percutaneous absorption through the palms and soles, which possess a predominance of eccrine sweat glands.

FACTORS INFLUENCING PERCUTANEOUS ABSORPTION

There are many factors which are thought to influence the rate and extent of percutaneous absorption. These will be discussed in this section.

Condition of the Skin.—The routes which a medicament may take in its passage through the intact skin have been discussed previously. If the skin is in an abnormal state, the limitations placed on the penetration of a medicament do not apply. If the barrier is destroyed by trauma, as in cuts, chapping, ruptured blisters, or in eczema, all substances pass freely into the dermis (9). Blank, *et al.* (18), have demonstrated by autoradiography that there is a striking increase in the penetration of sarin tagged with P^{32} following a superficial scratch extending just barely through the barrier. Similar data were obtained from trauma by adhesive tape stripping and puncture wounds.

Shelmire (33) pointed out that the vehicle of a topical preparation assumes more importance when the stratum corneum is intact and that differences in drug penetration attributable to the vehicle are more pronounced. On abraded or diseased skin there may be a large increase in both rate and extent of absorption of a drug in a vehicle (33).

It may be shown experimentally that the percutaneous absorption of materials coming in contact with the skin is significantly reduced in old age (34). This may be due in part to the atrophic changes of the pilosebaceous apparatus through which such substances are absorbed. It is a clinical fact that sensitization reactions to contactants are less commonly encountered in the older patient. In addition to lowered absorption, there may be a decreased capacity to form antibiotic conjugates in the skin or to form circulating antibodies.

Thermodynamic Properties of the Medicament.—The importance of the thermodynamic properties of a medicament as it relates to its percutaneous absorption has been discussed by Higuchi (35). These include the thermodynamic activity of the drug in the vehicle and in the skin barrier phase, and the diffusion coefficient of the drug in the vehicle and skin barrier phase.

Assuming that the vehicle containing the penetrating substance does not appreciably affect the skin, the following approximate relationship was set by Higuchi for an idealized system

$$\frac{dq}{dt} = (P.C.)\frac{(\text{concn. of drug})\,DA}{L} \qquad \text{(Eq. 1)}$$

where $(P.C.)$ is the effective distribution coefficient of the penetrating medicament between the vehicle and the barrier of the skin; (concn. of drug), the concentration of the medicament in the vehicle; D, the effective average diffusivity of the medicament in the barrier phase; A, the effective cross section area; and L, the effective thickness of the barrier phase. The main characteristics of the penetrating agent which determine its rate of entry through the skin, according to this equation, are its effective distribution coefficient and diffusivity in the barrier phase. The product of these two is referred to as the "permeability constant."

Actually, the important variable in the permeability constant of Higuchi is the effective distribution coefficient factor since the diffusivity of a substance of similar molecular weight and shape usually differs only slightly. The distribution coefficient, on the other hand, is an extremely sensitive function of molecular structure and size (35). Rothman (36) also emphasized the importance of the distribution coefficient of a penetrating substance between its vehicle and a membrane and between a membrane and its receptor solution on permeability. It depends chiefly on the force of molecular interaction between membrane and penetrating molecule. A substance can easily enter a membrane when the partition coefficient (membrane/vehicle) is high; it cannot easily leave the membrane when the partition coefficient (receptor solution/membrane) is low. In complex structures such as the skin, where the membrane may be nonpolar and the receptor-tissue fluids polar, a substance whose partition coefficient between polar and nonpolar solvents is nearly 1.0 will have the highest penetration rate. Thus, permeability is more rapid if there is some, but not too much, affinity between a penetrating molecule and a membrane. Under these conditions, the membrane will attract the penetrating molecule, but not so strongly that it fails to release it on the other side.

Higuchi (35) expressed Eq. 1 in another form in terms of the thermodynamic activity of the penetrating agent in its vehicle

$$\frac{dq}{dt} = \frac{A}{\gamma}\frac{DA}{L} \qquad \text{(Eq. 2)}$$

where a is the thermodynamic activity of the drug in its vehicle and γ is the effective activity coefficient of the agent in the skin barrier phase. Equation (2) shows the role of the activity of the drug in its vehicle while the properties of the base itself seem to play no part. For such systems the rate of percutaneous absorption measured for different ointment bases would be approximately constant provided the thermodynamic activity of the drug in the vehicles was maintained constant. Thus, all ointments containing finely ground suspensions of the drug

(thermodynamic activity equal to that of the solid drug) will produce the same rate of penetration. This again presupposes that the rate-determining step is essentially in the passage of the barrier phase. Since activities are more important than any absolute concentration, it is obvious that, for a given concentration of a penetrating substance, vehicles which have lower affinity (poorer solvent power) will normally produce faster penetration (35). For additional discussions on the absorption of medicaments from suspension and solution, readers are referred to works by Higuchi (35) and Wagner (37).

Effect of Moisture.—There are two opposite views on the influence of moisture on percutaneous absorption (38). Based on the concept that the skin is relatively impermeable to water, one school assumes that moisture on the outside has little to do with promoting percutaneous absorption. On the other hand, since it has been a long-standing clinical experience that watertight covering of the skin surface does influence percutaneous absorption, another school believes that moisture is a most important factor in promoting the passage of medicaments through the skin.

Renshaw (39) and Cullumbine (40) reported that much more severe lesions develop on human skin if mustard gas and nitrogen mustard gases are applied to wetted skin than to dry skin. Their work was interpreted by some to indicate that moisture plays an important role in promoting skin penetration. However, it is known that, in contact with water, mustards transform to various highly soluble, reactive, and toxic intermediary products before the inactive end products of the water-mustard reactions are formed (41, 42), and these may be responsible for the production of the severe lesions.

Laug, *et al.* (43), demonstrated that increased moisture promotes transfollicular absorption. They found that the absorption of mercury is increased fourfold by covering the site of inunction. It was assumed that the cover acts by interfering with the normal loss of moisture. Shelley and Melton (44) found that the application of histamine solution to the skin produced a prolonged effect when the site was covered, a result also probably due to the inhibition of water evaporation. Leslie-Roberts (45) concluded that moisture causes maceration of the horny layer and, therefore, provides a condition promoting the retention of substances in contact with the skin.

Recent work has more definitely established the importance of moisture in promoting percutaneous absorption. Higuchi (35) has stated that the transfer properties of several layers of the skin are probably strongly influenced by the presence of water since it is well sorbed by protein and protein degradation products contained in the outer skin. He demonstrated this type of behavior using artificial membranes. He has shown experimentally that, at very low humidity, the permeability of glyceryl monostearate is relatively insensitive to relative humidity, whereas near 100%, the rate of penetration is actually dependent on water activity. This is attributed to imbibition of water by the barrier phase exposed to saturated water vapor and consequent changes both in the diffusion coefficient and activity coefficient. Wurster and Kramer (46), investigating the absorption of three salicylate esters, demonstrated that a large increase in its absorption rate was produced by increased moisture conditioning. They assumed that percutaneous absorption involves a diffusional process, i.e., the spontaneous movement of a substance from an area of high concentration to an area of low concentration in the tissue fluids. They concluded that the magnitude of the effect of moisture on percutaneous diffusional rates has been shown to be proportional to the oil/water distribution coefficient and the water solubility of the test substance. The greatest increase of the penetration rate by moisture was found in the penetrant with the smallest oil/water distribution coefficient.

Shelmire (33) stated that hydration of the stratum corneum is one of the most important factors in the penetration of skin by a medicament. The hydration of the stratum corneum appears to increase the rate of passage of all substances which penetrate the skin. He (33) suggested that the mechanism was to increase the size of the pores. There will not only be a physical alteration of the tissue due to hydration but also at high water activities there will be changes in both the diffusion coefficient and activity coefficients of the penetrating agent (35). Shelmire (33) believes that the nature of the vehicle upon application and, when the water in it has evaporated, may markedly alter the activity of water in the stratum corneum.

Greases and oils are the most occlusive vehicles and induce the greatest hydration through sweat accumulation at the skin-vehicle interface (33). Emulsions of the water-in-oil type are somewhat less occlusive than greases (33). Oil-in-water emulsions tend to invert as the outer aqueous phase evaporates, and the final state may be considered to be a continuous oil film, containing other dissolved or suspended substances (33).

Humectants in such emulsions tend to decrease the extent of hydration of the stratum corneum by interference with the formation of a continuous oil film on the skin surface (33). Water-soluble vehicles produce the least change in hydration of the stratum corneum (33). Hydrophilic powders will decrease the extent of hydration by increasing surface area and, hence, increasing the rate of evaporation of water (33). Hydrophobic powders probably cannot retain moisture because they form a discontinuous film (33). All powders probably tend to interfere with the continuity of oil films and, in this manner, probably decrease the occlusive effect of any vehicle in which they are used (33). It should also be remembered that if an ointment is covered with a bandage there will be a tendency to hold perspiration and increased hydration; if left open to the air the perspiration can evaporate and hydration may occur (33). The thickness of the applied film of topical preparation will also directly affect hydration of the stratum corneum (33).

Effect of Vehicles.—There have been many studies on the role that a topical vehicle plays in the percutaneous absorption of drugs but until recently this subject has been somewhat in a state of confusion. This has been due in part to deficiencies in many of the methods used for measuring the extent of percutaneous absorption and to a lack of consideration of the thermodynamics involved in the interpretation of results. The thoughtful discussions by Higuchi (35), Shelmire (33), and Wagner (37) have clarified many aspects of this subject. They have thrown new light on the results obtained by workers in the field and indeed on the entire subject of percutaneous absorption.

It has been thought by a majority of workers in the field that the function of the vehicle in percutaneous absorption is to facilitate contact between the medicament and the absorbing cells. Absorption is best from vehicles which spread easily over the skin surface, readily mix with the sebum, and so bring the medicament into contact with the absorption cells. It was believed at one time that the primary factor influencing penetration through the skin was the vehicle itself. Thus the subdivision of ointment bases into epidermic, endodermic, and diadermic types became popular (47). It gradually became apparent that the thermodynamic activity and the diffusion coefficient of the medicament in the vehicle and skin barrier phase (35), the thermodynamic activity of moisture in the vehicle and skin barrier phase (35), and the degree of hydration of the skin (33) were of greater significance in influencing percutaneous absorption. It is believed that the vehicle itself is not capable of promoting the absorption of nonabsorbable drugs dispersed in it, but rather may modify the absorption properties of absorbable drugs (48).

In this section, some of the studies on the effect of vehicles on the percutaneous absorption of medicaments will be reviewed.

It has long been known that absorption of medicaments is better from animal and vegetable oils than from mineral oils because they readily penetrate the skin (49–51). Organic solvents like ether, chloroform, benzene, and acetone penetrate the skin with ease (52) and enhance the percutaneous absorption of a drug (53) to such an extent that toxic effects can occur. On the other hand, it has been demonstrated that defatting the skin with ether decreased the absorption rate of methyl salicylate (46). Higuchi (35) stated that the application of many solvents other than water appears to cause marked alteration in the resistance of the skin barrier toward penetration and that this phenomenon is possibly caused by marked changes produced by such solvents in the activity coefficient and diffusion constant of the penetrating agent in the skin barrier.

Strakosch (54) concluded from his histological studies that emulsion type bases do not have any effect on the degree of percutaneous absorption of salicylic acid. In contrast, results obtained from patch tests (1, 55), and analyses of urine (56, 57), and blood samples (58) obtained after topical application of ointment vehicles containing salicylic acid indicated differences in absorption from various emulsion-type bases. Similar contradictory results may be noted in percutaneous absorption studies of ointment vehicles containing sulfonamides (59–63).

Nogami, *et al.* (64), stated that the influence of the ointment base on the percutaneous absorption of salicylic acid was not great but that its absorption from hydrophilic ointment was less than from soft paraffin, simple ointment, and absorption ointment.

Bourget (65) and Kimura (66) made a quantitative study of the effect of different fatty bases on the percutaneous absorption of salicylic acid as judged by excretion of the drug. It was found that salicylic acid was rapidly absorbed and the amount depended on the vehicle; the ones giving the greatest absorption being lard and lanolin; the least effective, petrolatum.

Many workers have reported that the type of vehicle is of importance in the absorption of

mercury (22, 43, 67–69). However, Sollmann, *et al.* (70), have contradicted these reports.

Using radioactive iodine (71), radioactive sodium iodide (72), and potassium iodide (73), several investigators concluded that these substances appear to be more efficiently absorbed from wool fat than from lard. A difference in the type of emulsion vehicle had practically no effect on the absorption of the iodide.

Oil-in-water emulsions as well as water-soluble vehicles deserve special mention because of the claims that they would promote percutaneous absorption of drugs due to either the presence of surfactants or their water-miscible properties (43, 74). Eisner (75) demonstrated that a continuous oil phase would tend to reduce percutaneous absorption, a fact contradicted quite recently by the work of Gemmell and Morrison (60). There is no doubt, however, that a continuous water phase would be miscible with the secretions of the denuded and oozing surfaces of the abraded skin, and thereby promote the release of the medication. This was demonstrated by Zeutlin and Fox (76) who concluded that the release from water-soluble and oil-in-water type vehicles was far superior to the release from oleaginous and water-in-oil type vehicles. Strakosch and Clark (61) and Laug, *et al.* (43), supported with their experiments the theory of superiority of emulsion-type vehicles to oleaginous vehicles; they could not, however, show a significant difference in absorption for oil-in-water or water-in-oil emulsion vehicles while working with mercury (43) and sulfonamides (61). In contrast to this, a superiority of a water-in-oil vehicle to an oil-in-water vehicle was demonstrated by Gemmell and Morrison (60) who studied the absorption of sulfonamides.

It should be remembered that a great majority of percutaneous absorption studies have been carried out on animals. There may be a considerable difference in the rate and extent of absorption of a given drug in various species of animals and in man. The use of many types of test subjects is a further reason why results obtained from percutaneous absorption studies are somewhat confusing.

Effect of Surfactants.—Surfactants offer possibilities of improving topical vehicles and promoting a more thorough diffusion of the medicament from the vehicle, thus influencing therapeutic performance (77–78). Sweet (79) suggested that the mode of action for surfactants was that they are capable of emulsifying the sebum and, therefore, increase percutaneous absorption in this manner.

Duemling (74) reported that deeper and more rapid penetration of ammoniated mercury is obtained by the addition of a wetting agent to a paraffin ointment base. Meyers, *et al.* (80), investigated the absorption of potassium iodide and of phenolphthalein from various ointment bases through the intact skin of albino rats. They found no promoting action to be produced by the wetting agent, sodium tetradecyl sulfate.[1] MacKee (30) demonstrated penetration through intact skin of ferric chloride, bismuth (sobisminol), and sulfonamide from a series of topical vehicles called "penetrasols." These bases are composed of propylene glycol, antipyrine, xylene, and sulfonated wetting agents of the fatty acid esters of sulfosuccinic acid type. Shelley and Melton (32) confirmed the fact that these vehicles promote the percutaneous absorption of water-soluble drugs. In addition, they demonstrated that absorption occurred only from areas containing hair follicles. By analyzing the liver and kidneys of rats for mercury, Laug, *et al.* (43), compared the absorption of mercury from various ointment bases, some of which contained a surfactant, and found a significant increase in percutaneous absorption from the vehicles containing these agents. Duemling (74) demonstrated rapid and complete penetration of mercury from vehicles containing sodium lauryl sulfate. The incorporation of cholesterol in liquid petrolatum seemed to increase the penetrating powers of the vehicles (81), but the application of cholesterol to the skin appeared to reinforce the resistance of the fatty substances normally present in the skin and, therefore, reduced the percutaneous absorption of water-soluble drugs (82).

Stolar, Rossi, and Barr (83) detected a marked reduction in the percutaneous absorption of salicylic acid in hydrophilic ointments as the concentration of surfactants having polyoxyethylene groups was increased. They concluded that this is due to the complexing of the salicylic acid by the polyoxyethylene groups, i.e., salicylic acid has a low thermodynamic activity in these vehicles.

Stark, *et al.* (84), studied the influence of the nature of the surfactant in ointment bases on the release of various substances using an *in vitro* technique utilizing a membrane. Radioactive mercuric (Hg^{203}) and iodide (I^{131}) ions were incorporated into hydrophilic ointments emulsified with various concentrations of anionic, cationic, and nonionic surfactants. It was found

[1] Marketed as Tergitol 4 by Union Carbide Chemicals Co., 270 Park Ave., New York 17, N. Y.

that the ionic or nonionic nature of the surfactants substantially influenced the release. The nonionic-type surfactants promoted the release of both the iodide and mercuric ions to a greater extent than did the anionic or cationic types. Vinson and Choman (85) investigated the relative effects of surfactants on the cutaneous penetration of nickel sulfate through the skin of guinea pigs. They found that whereas the nickel sulfate did not penetrate when applied alone, sodium lauryl sulfate and sodium dodecyl benzyl sulfate did provoke its penetration.

Effect of Concentration of Medicament.—Strakosch and Clark (61) reported that increasing the concentration of sulfonamide from 1 to 10% in the vehicle caused no increase in their absorption when applied to the skin. It was similarly demonstrated by Laug, *et al.* (43), that the concentration of mercury in different ointment vehicles has very little effect on the degree of percutaneous absorption.

Higuchi (35) emphasized the role of concentration of a drug on its rate of absorption from a suspension-type ointment. He presented the following equation

$$\frac{dQ}{dt} = \sqrt{\frac{ADC_s}{2t}}$$

where A is the concentration of the drug expressed in units/cm.^{3}; C_s, the solubility of the drug as units/cm.^{3} in the external phase of the ointment; D, the diffusion constant of the drug molecule in the external phase; and dQ/dt, the rate of absorption. He concluded that the rate of release of drugs from such preparations can be regulated by controlling A, D, and C_s.

Effect of pH.—The rates of absorption of acidic and basic drugs are strongly influenced by the effective pH of the vehicle (35). The activity coefficient of the molecular form of such drugs is a rapidly changing function of pH for pH values greater than pKa for acidic compounds and less than pKw-pKb for alkaloidal drugs. Thus, as Higuchi states (35), the rate of absorption of histamine would be 10 times greater from a base buffered at pH 7.5 than from a base at 5.5, assuming that only the nonionized molecule is involved in the absorption process.

Bhatia and Barber (86) evaluated the effect of pH on the local anesthetic activity of benzocaine by measuring the pain threshold. They concluded that the maximum local anesthetic activity appeared to occur between the pH of 6 and 7, and decreased when the pH was varied. This was substantiated by Roques, *et al.* (87). Other workers (66, 88) concluded that systemic alkalosis increased the percutaneous absorption and excretion rates of various drugs while systemic acidosis decreased the rate of percutaneous absorption and excretion.

Miscellaneous Factors.—Other factors which influence the rate and extent of percutaneous absorption of medicaments include: the route of absorption (33, 35); the area to which the drug is applied (33, 35); the effective thickness of the skin barrier phase (35); and the individual contact time and frequency of reapplication (33).

It is well known that the elevation of skin temperature enhances percutaneous absorption. This may be due to a lowering of the viscosity of the sebum which would facilitate its mixing with the inuncted preparation. Furthermore, a rise in skin temperature also increases the cutaneous circulation through vasomotor dilation of the skin vessels. Blank, *et al.* (89), reported that the rate of penetration of sarin increases approximately twofold for a 10° rise in temperature. And finally, Higuchi (35) pointed out that the diffusion coefficient of a medicament, D, is inversely proportional to the viscosity of the vehicle.

METHODS OF MEASURING PERCUTANEOUS ABSORPTION

The results obtained by the various investigators who have studied the percutaneous absorption of drugs have been, for the most part, quite contradictory, probably due to inadequate and in many cases uncontrolled measuring procedures. There have been several excellent papers published in recent years in which the methods of measuring percutaneous absorption have been reviewed (3, 90–92).

Some authors classify the methods employed in measuring percutaneous absorption into *in vitro* and *in vivo* procedures. *In vitro* methods are of limited value but they are a means of assessing the ability of a vehicle to liberate the medicament under the conditions of the test. However, these methods can be, for the most part, dismissed as being quite useless in providing reliable information on percutaneous absorption. On the other hand, *in vivo* methods give a more accurate picture of percutaneous absorption, although the results obtained from such methods are often confusing.

Blank (3) has proposed a new classification of the various methods which have been used in studying percutaneous absorption. His classification will be used in this review.

Penetration into Models.—All measuring methods using models are of the *in vitro* type. They are of limited value but they are a means of assessing the ability of a vehicle or base to liberate medicament under the conditions of the test. Those reported are of a comparative nature and most are empirical, which prevents results from being compared with those from other techniques. Lockie and Sprowls (93) hold that the study of ointment bases by *in vitro* methods is essentially a study of diffusion rates.

The most common model which has been used to simulate skin is agar gel. It has been commonly used in studying the release of antibacterial agents from ointment bases. It has been too often assumed that those ointment bases which best release antibacterial agents to an agar gel will similarly release these substances to the skin when the ointments are placed on the skin. This is not so since the continuous phase in agar gel is water, and the skin is a much more complex chemical and physical structure.

Membranes composed of cellulose film (94) and sheep's bladder (95) have also been employed as skin models in *in vitro* procedures. While the assumption is made that the process of penetration in the skin is similar to the quantitative diffusion through a membrane, this does not make any allowance for differences in physicochemical properties of "dead membranes" and living tissue, the latter presenting a much more complex system, both physically and chemically.

Histological Studies.—A variety of procedures under this heading has been adopted to evaluate drug release from vehicles. Strakosch (96) has studied the penetration of salicylic acid, sulfur, resorcinol, and other substances by examining histological sections for evidence of keratolysis. No effort was made to measure such penetration quantitatively. Duemling (74) also utilized histological studies to measure the absorption of medicaments. Goldzieher, *et al.* (97), used histologic sections in their study of the penetration of estrogen. Histological studies made by Sobel, *et al.* (98), indicated that vitamin A is absorbed and utilized by the skin.

Use of Tracers.—Dyes and fluorescent materials have been utilized for a long time in percutaneous absorption studies. In recent years use has been made of radioactive substances as tracers in such studies.

Dyes.—One of the early methods used for studying penetration of substances into skin employed an oil-soluble dye to color the material being studied (99–102). Histological sections of the skin were then examined for the presence of dye. This method is subject to much criticism as one cannot be certain that the dye remains with the substance whose penetration is being studied.

Fluorescent Compounds.—The use of fluorescent compounds (100, 103) in penetration studies is open to the same criticism as those in which 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.

Radioactive Tracers.—The use of radioisotopes has become increasingly popular for studying percutaneous absorption (104). In such procedures an attempt is often made to synthesize the substance being studied so that it includes a radioisotope. Sometimes the molecular structure of the penetrating substance is modified somewhat by incorporation of the radioactive element, as for instance in the iodination of an unsaturated fat with I^{131} (100). Most of the percutaneous absorption studies involving the use of radioactive tracers are carried out by one of the following techniques: (*a*) direct counting at the surface; (*b*) autoradiographic technique; and (*c*) measurement of the radioisotope within the body.

The direct counting technique is performed by counting the amount of radioactivity on a given area of the surface of the skin by using an end window Geiger tube if the substance under study is an alpha- or beta-emitter of sufficient intensity. One such investigation (105) involved the application of 0.005 ml. of an alcoholic solution of thorium X, with an activity of approximately 2,000 disintegrations per minute, to four areas on the forearm. The activity on each of these areas was counted with an end window tube immediately following the application of the solution and 24 hours after application. The loss in activity represented the amount of thorium X which was absorbed. Ainsworth (92) described a method for studying percutaneous absorption of a radioactive compound by following its disappearance from the skin surface.

Autoradiographic technique, first developed by Axelrod and Hamilton (106), is a very useful procedure for demonstrating the penetration and localization of radioactive materials providing they emit alpha and beta rays. In order to prepare an autoradiogram, a biopsy must be made and tissue sections cut for transfer to nuclear track emulsions. The particular tech-

nique which is used will be determined by the type of radiation, the half-life, and the solubility of the radioactive isotope being studied. The penetration of thorium X (7, 107) and sarin (18) have been studied by this method and it was found that the penetration of the first is by way of the appendages and the penetration of the second is primarily transepidermal. Choman (108) has reported on an improved autoradiographic technique in which he measured the percutaneous absorption of sodium lauryl sulfate (S^{35}) and nickel chloride (Ni^{63}). An investigation of the pathways of absorption of hydrocortisone (C^{14}) free alcohol was carried out by the technique of autoradiography by Scott and Kalz (19).

In working with autoradiography one should be familiar with the following pitfalls. When a radioactive element that emits high energy radiation is being used, there will be a considerable scatter on the autoradiogram. Secondly, reducing substances in the tissues may react chemically with the emulsion and produce a shadow even when no radioactivity is present. Third, a shadow may be produced in the autoradiogram even when no radioisotope is present if the tissue and photosensitive emulsion are on separate slides which, during exposure, are clamped tightly together.

Percutaneous absorption has been studied by measuring the concentration of a radioactive substance which has been absorbed in the blood, urine, expired air, thyroid gland, and hair.

Hadgraft, *et al.* (109), have studied percutaneous absorption in rats by measuring the concentration of diiodofluorescein (I^{131}) in the blood. Hupf, Chase, and Barr (110) studied the effect of various ointment bases on the percutaneous absorption of salicylic (carboxyl C^{14}) acid through intact rabbit skin by measuring blood concentrations of the radioactive substance.

Goldzieher and Baker (111) investigated the absorption of estradiol 17-β (H^3) and progesterone (C^{14}) through the skin of guinea pigs by measuring the concentrations of the radioactive substances in urine. Hellman, *et al.* (112), Malkinson and Ferguson (113), Livingood (114), and Malkinson, *et al.* (115), studied the percutaneous absorption of topical corticosteroids (C^{14}) through human skin by measuring the concentrations of the radioactive substances in the urine.

Lange and Evans (116) applied an ointment containing radon to patients and the amount of radon in the expired air was taken as a measure of the amount passing into the bloodstream.

Cyr, *et al.* (117), and Skauen, *et al.* (72), studied the effect of ointment bases on the absorption of sodium iodide (I^{131}) through the skin of rats by measuring the concentrations of the radioactive substance in thyroid glands which were removed from the animals.

Edwards (118) has studied the absorption of topically applied amino acids in guinea pigs using methionine (S^{35}) by measuring the concentration of radioactive material in newly grown hair.

Measurement of Physiological Reactions.—The percutaneous absorption of certain drugs in ointment form has been studied making use of their pharmacological effects or end points. Those which have been used include a rise in blood pressure (119), measurement of vasodilation (120), red tear response (121), production of anesthesia (122), reduction in pain threshold (123–125), production of convulsions (126), production of urticaria (127, 128), vasodilation (129), and death (122, 129).

The effect of passage of hormones through the skin has been the subject of wide research; a few of the many references will be quoted. Different criteria have been used for the measurement of percutaneous absorption of hormones. These consisted of the growth of testes (130), development of a scrotal hernia (131), mammary growth (132, 133), the increase in weight of the seminal vesicles and the prostate gland (134, 135), increase in growth rates of feathers and comb of a capon (135), and the ejaculation reflex as well as the weight of the ejaculate (133, 136).

Among the methods used for studying the percutaneous absorption of vitamins may be mentioned those based on the dark adaption test for vitamin A (137), the thickening of skin in rats by vitamin A (138, 139), weight gain and increase in growth caused by vitamin A in vitamin A-deficient rats (98, 140, 141), and curative effects in man for vitamin C (142), vitamin D (143), and panthenol.

Analysis of Tissues: Skin, Blood, Urine, etc.—Probably the most common method of studying percutaneous absorption *in vivo* is by analysis of the circulating blood (58, 60, 110, 144), urine (145–150), and feces (151) at different intervals following application of the material to the cutaneous surface. One of the difficulties encountered in attempting to estimate quantitatively the amount of material which has penetrated the skin by analyzing the blood, urine, or feces is the fact that the rate at which material leaves the blood stream and is excreted or stored in the tissues is seldom known.

In those cases where drugs appear to have a greater affinity for a particular organ, the organ may be excised and analyzed for drug content. This principle has been used by some workers for the determination of the percutaneous absorption of mercury (43, 152–154), iodides, and iodine (117, 155, 156).

Loss of Penetrating Substance from the Surface.—If a known amount of a nonvolatile substance which has been allowed to remain on the cutaneous surface for a definite period of time is quantitatively removed from the surface and analyzed, the difference between the amount applied and the amount removed may be thought to have penetrated the skin (157). Blank (3) discusses several pitfalls in this method. Quantitative removal is difficult. Some materials combine chemically with the constitutents of the cornified epithelium and then cannot be easily separated from it although they have not penetrated into skin any further than the stratum corneum. The amount of most substances which penetrates is often so small that errors in analysis may be of the same order of magnitude as the amount absorbed and, therefore, one can never be sure that the difference between "before" and "after" determinations truly represents the amount of material which has penetrated. Now that radioactive substances can be prepared it is possible to determine loss from the surface simply by determining the decrease in radioactivity. This subject has been discussed previously.

PERCUTANEOUS ABSORPTION OF MEDICAMENTS

The consensus of the majority of investigators who have studied the percutaneous absorption of medicaments appears to be that the greater the solubility of the drug in oil than in water, the greater its percutaneous absorption. However, it should be emphasized that some degree of solubility of a medicament in both oil and water is thought to be essential for it to be absorbed percutaneously. This phenomenon is not specific to percutaneous absorption, but holds true to absorption and action of drugs in general (158).

The following drugs, all more oil-soluble than water-soluble, have been reported to be absorbed percutaneously: benzocaine (86), iodine (117, 145, 155, 156), phenolsulfonphthalein (159), sulfathiazole (160), sulfanilamide (60, 61), sulfadiazine (161), ammoniated mercury (67), mercuric oxide (43), salicylic acid (45, 54, 58, 83, 153, 162–164), methyl salicylate (163), glycol salicylate (163), diiodofluorescein (109), pyribenzamine (165), morphine (166), strychnine (126, 166), pilocarpine (166), aconitine (126, 166), acetylcholine oleate (167), eserine (121), nicotine (129, 166), stearic acid (100), mustard gas (40, 101), phenol (168–172), and sarin (18, 89).

Although it is thought by many that predominantly water-soluble drugs are not absorbed percutaneously, there are many references in the literature which report that such absorption does take place. Among the water-soluble drugs which have been proved to be absorbed percutaneously are: tritium oxide (173, 174), deuterium oxide (175), sodium chloride (146, 176), thiamine hydrochloride (12), vitamin C (12), mercury bichloride (177), morphine sulfate (166), strychnine sulfate (12), pilocarpine nitrate (32), acetylcholine chloride (32), epinephrine hydrochloride (32), histamine phosphate (32), sodium iodide (71, 72), potassium iodide (71, 178, 179), pyribenzamine hydrochloride (180), penicillin G potassium (181), chlorcyclizine hydrochloride (182), and sodium salicylate (6, 183–186).

Much work has been carried out in recent years on the percutaneous absorption of vitamins, corticosteroids, and sex hormones, and these will be reviewed.

Vitamins.—The percutaneous absorption of vitamins has been reviewed recently by Rubin (187). In brief, vitamin A has been shown to be absorbed percutaneously in animals (53, 140, 141, 189–191) and in man (137, 191). Vitamin D has been shown to be absorbed percutaneously in animals and humans (192, 193); vitamin K in infants (194); and vitamin E in the rat (195). All four of these vitamins are oil-soluble and this property undoubtedly is largely responsible for its absorption.

Of the water-soluble vitamins, panthenol has the largest literature concerning percutaneous absorption. Absorption has been demonstrated in animals (196, 198) and in man (198, 199). The percutaneous absorption of vitamin B_6 (200, 201), thiamine hydrochloride (200, 202), riboflavin (200), calcium pantothenate (200), and vitamin C (203) has also been reported.

Sex Hormones.—The effectiveness of estrogens and progesterones in dermatology and in cosmetics is based on their percutaneous absorption. The rate of absorption of these substances is primarily determined by the vehicle (204).

The most widely used hormones in cosmetics are the estrogens. These substances have produced demonstrable results in the treatment of acne in both sexes. Topical estrogens were also

observed to have a restorative effect on distorted elastic fibers common in aging skin. Among others, Masters (205) and Goldzieher and Baker (206) have demonstrated the percutaneous absorption of estrogens.

Progesterone has been reported to penetrate the intact skin rapidly and with great ease (206, 207). The absorption of progesterone appears to be more rapid than with estradiol (206). Both testosterone and its propionate ester are absorbed through the skin, the free alcohol being more effectively absorbed than the ester (208).

Corticosteroids.—Much interest has been shown in recent years on the percutaneous absorption of the corticosteroids. This subject will be reviewed here.

Earliest studies to demonstrate the percutaneous absorption of hydrocortisone were based either on the physiological effects it produces or an increase in its metabolites in plasma or urine. Smith (209) found no significant change in the circulating eosinophile count following the application of 150 mg. of hydrocortisone acetate to normal or clinically inflamed skin of patients. In another investigation (210), this author failed to note any changes in the urinary excretion of 17-ketosteroids and 17-hydroxycorticosteroids in similar-type subjects. Witten, *et al.* (211), studied the blood and urinary levels of 17,21-dihydroxy-20-ketosteroids in humans for 3 days in response to external application of hydrocortisone acetate-containing ointments without finding significant changes. In another study, Fitzpatrick, *et al.* (212), could not demonstrate the absorption of hydrocortisone acetate in a lotion vehicle following its application to the skin of patients. As a result of these studies, it became apparent that for humans there was little danger from the absorption and consequent systemic effects of topical hydrocortisone in concentrations of 1 to 2.5%.

The first direct demonstration of the percutaneous absorption of hydrocortisone was carried out by Malkinson and Ferguson (113) who demonstrated the radioactive 17-ketosteroid fraction in the urine following the topical application of hydrocortisone (4-C^{14}) to normal human skin in the form of an ointment. Livingood, *et al.* (114), confirmed the absorption of hydrocortisone (4-C^{14}) in humans. Liddle (213) and Paulsen and Rerup (214) also demonstrated the percutaneous absorption of hydrocortisone in humans.

Although it had been shown that hydrocortisone penetrates the superficial barrier (19), Malkinson (215) found that removal of the barrier layer tremendously enhances the penetration of this hormone.

Investigations of the type previously described for hydrocortisone have been carried out with cortisone. Danto and Maddin (216) did not observe any change in the number of circulating eosinophiles in humans after the topical application of cortisone acetate. Malkinson and Ferguson (113) detected the radioactive 17-ketosteroid fraction in the urine following the topical application of cortisone (4-C^{14}) to normal human skin in ointment form. No increase in the penetration of cortisone (4-C^{14}) acetate occurred upon application to stripped sites (215).

Fitzpatrick, *et al.* (212), reported five cases of increased weight and ankle edema in patients treated with 0.2% fluorohydrocortisone acetate lotion. Livingood, *et al.* (217), found edema, weight gain, and/or diminished urinary excretion of sodium in a group of 11 patients receiving topical fluorohydrocortisone. Although it is apparent that fluorohydrocortisone is absorbed percutaneously, as yet there is no decisive evidence of its penetration through normal skin.

There has been very little carried out on percutaneous absorption studies of other corticosteroids.

SYSTEMIC TOXICITY RESULTING FROM PERCUTANEOUS ABSORPTION

Investigations for potential systemic toxicity resulting from percutaneous absorption must be considered as simply one aspect of the overall evaluation of safety which is carried out to determine whether products are safe for patient use (218). The systemic toxicity resulting from the absorption of medicaments applied to the skin is influenced by two independent properties: (*a*) the ability of the medicament to penetrate the skin and (*b*) the inherent toxicity of the medicament toward the patient. Systemic toxicity can occur only when the medicament in question is both absorbed and toxic. However, dangerous toxic symptoms may result on application of potent medicaments to large areas of broken skin. Also of importance are the concentration of medicament and the area of broken skin to which it is applied.

Systemic toxic reactions are important from the standpoint of both acute and chronic effects. Acute toxicity is easier to measure and the results are easier to interpret. On the other hand, in studying the chronic effect of a medicament, it is important to relate the induced changes to the dosage and the frequency of application to the subject. On this basis, the possibility of a similar human exposure may be drawn.

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