

Water and the Skin

BERNARD IDSON, Ph.D.*

Presented in part April 5, 1972, before the New York Chapter, Clifton, N.J.

Synopsis—Problems of dry SKIN are those of inadequate hydration of the stratum corneum. WATER is continually diffusing outward from sweat glands and transepidermal diffusion as long as the relative humidity remains less than 100%. HYDRATION dramatically increases the permeability of the skin. Yet, even the hydrated stratum corneum is an effective BARRIER against water loss. Diseased skin is more permeable to water loss. The whole stratum corneum is probably the principal skin barrier to water. Agents that increase the permeability of the skin, such as DMSO or surfactants, damage or alter the nature of the stratum corneum reducing the diffusional resistance. Diffusion through the stratum corneum is passive, influenced chiefly by the water vapor pressure gradient. Much of the water-binding capacity of the corneum is due to the presence of hygroscopic water-soluble substances, which appear to be protected by lipid material which must be removed with solvents before they can be extracted by water. The osmotic properties of the horny layer suggest there is a semipermeable membrane system which prevents the water-soluble substances from being washed out when the corneum is immersed in water but does not prevent the hygroscopic substances from holding water in humid atmospheres. The membrane system may be the CORNEUM CELL WALL.

INTRODUCTION

Water plays a unique role in the dry skin condition. The lack of adequate water in the upper layer of the skin, the stratum corneum, results in dry and chapped skin (1–3). A level of between 10 and 20% water is needed to maintain normal softness and pliability of the skin (1, 2). The normal supple appearance of skin is due in large part to the capacity of the horny layer to bind water. The skin presents a formidable “barrier” to water loss and this is probably due to the whole stratum corneum.

When the relative humidity is low, the amount of water diffusing into the stratum corneum from the underlying tissues is insufficient to replace the water lost to the atmosphere and chapped skin results (4, 5). The water deficiency in the cornified epithelium can only be remedied by restoring the normal water content (2, 6). Fats and other oily materials do not aid this dry

*Hoffmann-La Roche Inc., Nutley, N. J. 07110.

skin condition unless they are sufficiently occlusive to reduce the water loss into the air and permit the buildup of moisture in the skin (6, 7). The problems of dry skin are those of adequate transport of water into the horny layer and prevention of gross water loss.

Dry stratum corneum presents a high resistance to penetration of water and water-soluble substances. Hydration dramatically increases the permeability of the skin (1, 2, 6-42), as well as affecting its mechanical properties (1, 2, 13, 43-48). This review is concerned with the nature of the hydration of the skin, water binding, and the "barrier" zone.

HYDRATION

The state of hydration of the stratum corneum is dependent on the rate at which water reaches the corneum from the layers beneath it, the rate at which water leaves the skin surface by evaporation, and the ability of the corneum to hold moisture (49).

The rate at which water reaches the skin surface is governed by the supply of water from the eccrine sweat glands and moisture obtained by transepidermal transport. Secretion from sweat glands consists mainly of water and electrolytes, and would therefore be expected to influence the water content of the stratum corneum by inducing skin hydration. It is estimated that a total transepidermal water loss of about 85-170 ml/day occurs from an average adult under average conditions. In such conditions, at rest and without visible sweating, sweat secretion amounts to about 300-500 ml/day, a substantial proportion of which is from the palms and soles. Transepidermal or upward diffusion of water yields smaller amounts of water since it is limited by the low water vapor permeability of the stratum corneum itself (50). Hydration of the skin varies with changes in the relative humidity or moisture saturation of the air and not the absolute amount of moisture in the atmosphere (5, 13). The degree of hydration is never constant as the variable environmental relative humidity alters the amount of water reaching the surface of the skin. Hence, water is constantly diffusing from the lower cells of the epidermis. As long as the ambient relative humidity remains less than 100%, water is continuously transferred outward. Under normal conditions, therefore, a gradient in water concentration exists within the stratum corneum. The activation energy for the diffusion of water through fully hydrated stratum corneum is 13-16 kcal/mole (14, 20). Through dry tissue it is most likely larger since hydration swells and softens the tissue.

Hydration increases the thickness of the corneum several fold, as it changes from a tissue which normally contains 5-15% water to one which may contain as much as 50% water (10). Upon immersion in water, swelling of the corneum starts in a few minutes and continues up to 3 days. The tissue can absorb up to 5-6 times its weight when fully hydrated and this water is strongly bound within the intracellular keratin (19, 51, 52).

There is little to no correlation of age and water loss. However, in a study of human fetal skin it was found that in the early stages of pregnancy the skin is very permeable to tritiated water. During this time the skin may serve as a major pathway for solute and water exchange between amniotic fluid and the fetus. However, as the fetus increases in age a large decrease in skin permeability to tritiated water occurs (54).

Regardless of the increase in permeability, the highly hydrated horny layer remains quite water-impermeable and represents a stable and extremely effective diffusion barrier in comparison with the viable skin layers (28). Hydrated stratum corneum has a diffusional resistance almost 10,000 times greater than an equivalent layer of water (55). The activation energy and entropy of diffusion show that the mobility of water within the tissue is orders of magnitude less than in liquid water and suggest that the diffusing unit is larger than a single water molecule (28, 56).

Measurements of the evaporation rates from stripped and nonstripped skin may be used to calculate the resistance of the stratum corneum to water permeation (57). The resistance of normal human forearm skin to water transport is in the range of 150–600 sec/cm. The resistance of the same "stripped" skin was in the range of 0.4–1.25 sec/cm (57). The water transmission rate of fish and frog skins approximates free evaporation of water and is 6–20 times greater than mammalian skins (58).

The resistance of hydrated stratum corneum is illustrated by a comparison of the diffusion coefficients of drugs in water and in human stratum corneum. Diffusion coefficients ranging from 10^{-9} to 10^{-13} cm²/sec could be expected for drugs penetrating hydrated stratum corneum. The diffusion coefficient in water for most drug molecules would be expected to range from 10^{-5} to 10^{-6} cm²/sec (55). Hence, the diffusion of water and low-molecular-weight, water-soluble molecules through hydrated keratin is much more difficult than the corresponding free diffusion in aqueous solution. The filament-matrix ultrastructure is preserved under hydration and the water appears first to enter between the filaments and only later to diffuse within them (59). The transepidermal water loss by passive diffusion varies between 0.2–0.6 mg/cm²/hr dependent on the conditions of temperature, humidity, air flow, and body site measured (7, 11, 12, 14, 53, 57, 60–63). With regard to body sites, Bettley and Grice (61) found a transepidermal water loss of 0.35 mg/cm²/hr on the front of the abdomen as compared with 0.6 on the thigh.

Diseased skin in which the process of keratinization is disturbed becomes more permeable to water loss (53, 64, 65). Skin affected by eczema and psoriasis could be 8 to 10 times more permeable than normal (64). Even the apparently normal skin of eczematous subjects shows a higher rate of water loss than normal. When large areas of the body surface are so affected, the whole-body skin water loss may reach as much as 2 l. daily. Comparative studies showed that when compared to the normal, ichthyotic skin showed

twice the normal loss. In erythroderma the water loss was 14 times the normal (53). This is quite apart from sweating and is essentially outside the control of body mechanisms. Apart from its effect on water and salt metabolism, the evaporation of this amount of water from the skin surface requires over 1000 calories, a formidable daily energy requirement of patients who are ill (60).

The state of hydration of the stratum corneum has been accepted for quite some time as of major importance in influencing percutaneous absorption. Wurster and Kramer (16) measured the rate of penetration of esters of salicylic acid through skin with dry and hydrated stratum corneum. They found that 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 (27) showed the dual importance of these factors on the penetration of excised skin. Full hydration of the keratin, accomplished by layering water over acetylsalicylic acid on the epidermal surface, dramatically increased the penetration when compared to conditions of lower humidity at the same temperature. The importance of hydration can also be found in those investigations employing occlusive plastic film in steroid therapy. Here, the prevention of water loss from the stratum corneum and the subsequent increased water concentration in this skin layer enhances the penetration of the steroid (23, 29–32). McKenzie and Stoughton (29) have shown that penetration of corticosteroids may be increased 100-fold by occluding the site of application and thus hydrating the stratum corneum. It is also quite probable that some drugs increase the degree of hydration of the skin. This is most likely to occur with substances that readily penetrate the skin, and thus reach sufficiently high concentrations to produce an osmotic effect. A few steroids, such as estrogens and pregnenolone, have been reported to produce a beneficial effect on aging skin by hydrating the skin (66, 67).

Topically applied vehicles have the potential to either increase or decrease the quantity of water in the horny layer. The efficiency of varied type vehicles in aiding penetration can be reasonably predicted on the basis of their effect on hydration of the stratum corneum or how the vehicle alters the activity of water in the stratum corneum and influences the stratum corneum/vehicle partition coefficient. Water-insoluble, oily materials such as petrolatum, lanolin, and isopropyl myristate significantly retard the rate of loss of water from the skin surface. On the other hand, certain emulsifiers and humectants increase the rate of moisture loss (7, 68–70).

Skin keratin has an isoelectric point of 3.7–4.5. Hence, it would appear that hydration would be affected by changes in pH. Yet, there are little data to support the idea that the pH of vehicles within the range tolerated without immediate irritation or skin damage affects the hydration state of skin greatly (16). Studies of stratum cornea from varied species (13, 15, 71) showed very little change in the swelling or hydration characteristics of skin between pH

values of 1–10. Significant increases in water diffusion rates and decreases in water-binding capacity were observed with buffer solutions above pH 10, and primarily at pH 12. These changes at high pH values can be attributed to extraction of water-binding substances and the solubility of keratin in the pH range 10–12.

Diffusion of water through the stratum corneum is a purely passive process affected only by physical factors as determined by ambient conditions, chiefly the water vapor pressure gradient across it (7, 8, 10, 72–77). The permeability constant of water in the skin at 25°C is approximately 0.5×10^{-3} cm/hr, corresponding to a flux of 0.2 mg/cm²/hr (5). The flux is approximately the same whether liquid water or saturated water vapor is applied *in vitro*. These data suggest that the surface concentration, which is not very different in these two instances, controls the diffusion rate and this is good evidence that water transport through skin is a passive process (10). There is little or no evidence to support specialized active transport for cells of the stratum corneum. In order for water to move against a concentration gradient, an energy yielding “pump” is predicated in a viable epidermis. It certainly 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. It appears that the major route of absorption of water during steady-state diffusion is transcellular through “boundwater” regions in the hydrated stratum corneum (78, 79). The practical and theoretical considerations involved in studying water transport through the stratum corneum are analogous to those of studying transport through monomolecular layers (57). However, the mechanism of transport through hydrated stratum corneum may be quite different from that through normal stratum corneum. The low diffusion constant and high activation energy obtained for water suggest that extensive hydration does not drastically affect the “barrier” function of the stratum corneum (20). The more important point to consider is the thermodynamic activity of water in the barrier phase, not just the amount there.

WATER BINDING

The normal appearance of skin and its ability to resist environmental irritants, both physical and chemical, are attributable to a large degree to the capacity of the corneum layer to bind water (13). The stratum corneum has a natural ability to hold a certain amount of water against the diffusion gradient created by adverse atmospheric conditions such as low dew points (7, 44).

Most of our information on water-binding results from interpretation of solvent extraction of the skin. Little change occurs in either the water-binding capacity in a humid atmosphere or in the weight of human callus when it is extracted with water or with polar lipid solvents such as pyridine. How-

ever, there is a significant decrease in both the weight and the water-binding capacity when callus is extracted with water after the solvent treatment although the callus can still hold considerable quantities of water (1). The loss in weight is due to the extraction of a water-soluble fraction which markedly enhances the ability of the stratum corneum to hold water. The corneum contains about 25% of its dry weight of these water-soluble substances (11, 80, 81). It seems well established that much of the water-binding capacity of corneum is due to the presence of these hygroscopic water-soluble substances and that they are in some way protected by a lipid material which must be removed with solvents before they can be extracted by water (13, 24, 44, 49, 80-87).

There is no apparent correlation between the loss of water barrier capacity of the skin and the total amount of lipid extracted from the skin by the varied solvents (88). Lipid solvents appear to have very little effect on the structural elements of the stratum corneum. Neither the mechanical strength is changed nor is any change detectable in the birefringence after lipid extraction. On the other hand, large amounts of lipid material are removed and open membrane interstices are apparently formed which act as low energy diffusion pathways. The lower activation energy for diffusion and nonselective higher permeability for all solutes indicates liquid-like transport through these solvent-filled interstices. The capacity of the tissue to bind large amounts of water is also destroyed by treatment with delipidizing solvents. This suggests that the lipid material does not simply plug the interstices in the membrane but rather aids in stabilizing the water structure in concert with the other tissue components (28).

The degree of alteration of water barrier capacity varies greatly among different specimens of skin extracted by the same lipid solvent for the same period of time (15). Water transmission was measured through isolated human, rabbit, fish, and frog skins (89). The rate of transmission was much greater for fish and frog skin. Human and rabbit skins contain a hexane-soluble lipid which appears to be the major regulator of passive water holding. This hexane-extractable lipid was not detected in the fish or frog skin. Burned mammalian skin contained 30% or less of the normal water-holding lipid and transmitted up to 4 times more water than intact skin. Water transmission by the skin surfaces was inversely proportional to the water-holding lipid content of the surface.

The effect of relative humidity on water binding and extensibility indicates a close relationship between the two. It is interesting to note, however, that it is that fraction of the water which is held by the water-soluble substances which is responsible for most of the extensibility. Removal of the water-soluble substances with ether followed by water extraction results in a very large reduction in extensibility while there is only a comparatively small reduction in the water-binding capacity. The residual water which is bound by the sol-

vent-extracted corneum in humid atmospheres does not appear to contribute significantly to the extensibility (44). No change in elastic modulus is obtained if the corneum is extracted only with water (lipid left intact), but the elastic modulus increases significantly if the callus is treated with chloroform or detergent solutions followed by aqueous extraction (45).

The osmotic properties of the stratum corneum itself depend upon the presence of water-soluble substances within the corneum and suggest that there is a semipermeable membrane system operating within the corneum. The loss of osmotic properties after extraction with solvents indicates that the semipermeable membrane system contains lipids, the removal of which alters the permeability of the membrane system so that the water-soluble substances responsible for exerting the osmotic forces can escape. The membrane system prevents the water-soluble substances from being washed out when the corneum is immersed in water but does not prevent the hygroscopic constituents from holding water in humid atmospheres (44).

The presence of cholesterol and phospholipid in solvent extracts of guinea-pig footpad corneum suggests that the membrane system protecting these water-soluble substances from dissolution in water is the corneum cell wall. The effect of powdering the corneum in liquid nitrogen shows that breaking the cell wall allows water to extract the water-soluble substances and reduce the water-binding capacity. Breaking the cell wall has the same effect as extraction with solvents. The evidence is therefore consistent with the idea that the semipermeable membrane system is the cell walls of the individual corneum cells (44).

The hygroscopic substances have been grouped together and called the "natural" moisturizing factor (NMF) (82). They consist of a mixture of amino acids, organic acids, urea, and inorganic ions. Sodium lactate (24) and the sodium salt of 2-pyrrolidone-5-carboxylic acid (86) appear to be the most hygroscopic components of the natural mixture. Collectively, their hygroscopic properties have been found to be approximately equal to those of glycerine (85).

Relatively little is known as to how the hygroscopically bound water plasticizes the corneum proteins which are responsible for its mechanical integrity. Park and Baddiel (45) postulate that aqueous plasticization of the corneum is due to direct protein hydration both in the presence or absence of hygroscopic substances. Due to their ionic or dipolar nature, the hygroscopic substances will be bound to the proteinaceous components of the stratum corneum. Location of these bulky molecules between protein chain segments will create a more open structure than would exist in their absence, leading to enhanced protein hydration. Upon removal of the hygroscopic molecules the protein matrix collapses with the resultant formation of more protein-protein bonds (mainly hydrogen bonds and ionic interactions). The higher modulus values obtained for the hygroscopic-free corneum samples result from the

rigidity that the additional bonds formed (after removal of the hygroscopic substances) impart to the structure. Due to the reduction in swelling capacity of the corneum imposed by this structural collapse, water uptake will also be less. This is also indicated by water-binding isotherms (1, 13) and differential thermal analysis (90).

Indirect evidence for the cell membrane system rather than the keratin being the rheologically active material in the stratum corneum is provided by the fact that isolated keratin films swell considerably in water, almost to the point of dissolution, and display no detectable elasticity. This behavior contrasts with that of untreated and extracted corneum, both of which have finite elastic modulus values in water (45). The concept that the mechanical strength of the corneum resides in the cell membrane system is supported by the fact that the cell membrane protein (110) has a much higher cystine content ($\times 3-4$) than the keratin filaments (91) leading to more permanent (with regard to disruption by water) disulfide crosslinks in the membrane (45).

WATER BARRIER

Transepidermal water loss is a small fraction of the water loss from a comparable area of water. The skin thus has the property of a water barrier, and the stratum corneum is generally recognized as representing the principal skin barrier to water loss as well as to entry of environmental noxious agents (6, 7, 60). Removal of the skin barrier by cellophane tape stripping will enhance the absorption of almost any substance.

The barrier possesses a resistance to the transport of water molecules, and it is irrelevant whether these water molecules are passing from the inside to the outside of the skin or *vice versa*. It is also irrelevant whether the water is in the liquid or in the gas phase on one or both sides of the skin (57). The vapor barrier properties of the stratum corneum are virtually mechanical and not dependent on living cells, functioning enzyme systems, intact cell membranes, or attributable to an "electrical double layer" (1, 7, 8, 12, 14, 88, 92).

The location of this epidermal barrier has been the subject of considerable controversy. At one time it was thought that the barrier layer resided between the stratum granulosum and stratum corneum (92-94). Later it was placed in the lower portion of the corneum (1, 95, 96). However, at present, the available data offer no evidence for the concept that different layers within the main bulk of the stratum corneum have different diffusivities (10). Water permeability experiments on isolated stratum corneum (8) indicate that the barrier to penetration is the entire stratum corneum (10, 15, 50, 54).

Water diffusion rate can be principally used to evaluate the functional state of the skin barrier. It is probably the most sensitive measure of barrier intactness (97). If the skin barrier is removed, water will evaporate from the skin surface at approximately the same rate as from a free water surface. Water

permeability is measured in terms of evaporative loss from an epidermal surface covering the aqueous phase. Most methods depend upon the use of two chambers, in one of which is placed a known quantity of the substance under study, separated from a second chamber by a piece of whole skin or separated epidermis. The amount of the substance recovered from the second chamber is measured (10, 60, 98–100).

Barrier renewal is a complex process. After loss of the stratum corneum, a temporary barrier is formed by rapid conversion of granular cells into parakeratotic cells. As the parakeratotic layer thickens water loss gradually decreases. The temporary barrier persists until the regenerating epidermis becomes capable of forming normally keratinized cells (21,101). The reformation of the barrier to increased sensible water loss is very rapid. Three days after the injury, the water vapor loss of the skin has been restored to such an extent that the difference between the water vapor loss of the damaged and normal skin has become very small. Total regeneration requires about two weeks (95, 101–103).

There is limited knowledge of the composition of the barrier. However, it can be equated with that of the corneum. The main cellular components are proteins, lipids, and water combined into an ordered structure. The cells are bound into a layered membrane which is *ca.* 15 μ thick when dry but increases to *ca.* 48 μ when fully hydrated (104). When hydrated, the stratum corneum contains approximately 75% water, 20% protein, and 5% lipid.

The sebaceous secretion, sebum, forms an irregular film on the surface of the skin, which has been estimated as averaging 0.4–4 μ thick (73). Sebum does not contribute to any significant effect to the epidermal barrier. Removing the sebum by mild swabbing with acetone or ether has no effect on the rate of water loss (105) and adding up to 10 times the normal amount (30 μ thick) has only a slight effect (106). The sebum apparently does not form a functionally complete lipid layer over the skin, leaving gaps which allow access to the underlying stratum corneum (107). It is nonocclusive and relatively porous due to the presence of branched-chain aliphatic compounds (87). However, the oil does help retain the water on the surface of the skin.

Frequent washing with soaps and detergents, which may dissolve the oil, can cause loss of the water-holding capacity of the epidermis. While the surface lipids offer little resistance to the passage of compounds, studies of removal of lipids from the cutaneous surface (2, 7, 16, 69, 88, 109) indicate that lipids participate in epidermal water function. Onken and Moyer (7) showed that barrier function is restored when extracted lipids are returned to the skin. This 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 (88) applied a number of solvents to the epidermal side of hairless mice and human skin. All of the 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.

There are a number of agents that increase the permeability of the skin by reducing the epidermal barrier to water transpiration. All of these agents must have one common property, the ability to damage or alter the nature of the stratum corneum in such a way that its diffusional resistance is reduced (6). For example, the activation energies for water through epidermal membranes, pretreated with a mixture of chloroform-methanol (2:1) to delipidize the membrane, range from 6.08–6.5 kcal/mole (29). These values are much lower than the activation energy of approximately 15 kcal/mole (20) for water penetrating normal epidermis and undoubtedly reflect substantial membrane damage.

Solvents with both polar and lipophilic properties apparently 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 dimethylsulfoxide (DMSO), dimethylformamide (DMF), and dimethylacetamide (DMA) all have the ability to produce a striking but reversible suppression of barrier resistance. They are all strongly hygroscopic and it is likely that the presence of these substances in the stratum corneum increases the hydration of the tissue and therefore its permeability. DMSO has a permeability constant of 300 $\mu\text{cm}/\text{min}$ (110) as compared to a value of 45 $\mu\text{cm}/\text{min}$ for water through excised human skin (73).

DMSO appears to alter the complex interrelationship of water, lipids, protein, and mucopolysaccharides regulating epidermal water barrier function (111). This may explain, in part, the sometimes observed variable role of DMSO. Swelling may induce the formation of channels within the matrix of the stratum corneum which either favor the passage of varied compounds (112) or lower the diffusional resistance of the stratum corneum. DMSO can extract soluble components from the stratum corneum, suggesting ultrastructural modifications consistent with an increase in permeability. There are possible reversible configuration changes in skin protein structure brought about by substitution of integral water molecules by DMSO, with resultant swelling (113). A sharp increase was found in the flow rate of tritiated water through hairless mouse skin if the DMSO solutions used to pretreat the skin contained in excess of 70% DMSO. Low concentrations appear to produce little change in the skin barrier, whereas marked effects are observed in solutions containing 50% DMSO, or more (114).

Polar, hydrogen-bonding solvents like water and DMSO are capable of reacting with the bulk of the tissue (the protein) and not just the minor lipid component. When applied in large concentration, they become incorporated into the tissue and constitute a large percentage of the membrane substance. The membrane expansion is determined by the extent of their incorporation

and the accompanying reaction of the structural elements of the tissue. The membrane diffusivity appears to be determined by the stability of the resulting hydrogen-bonded solvent structure, for it is largely the solvent in the membrane through which diffusion occurs. The membrane-water association is apparently much tighter than the membrane-DMSO structure and the diffusivity of the membrane-water media is accordingly much lower (28).

Soaps and detergents are perhaps the most damaging of all substances routinely applied to the skin (10, 115). Treatment of isolated human or animal callus with soap or detergent solutions can reduce its ability to hold water in a humid atmosphere (13, 43). Dilute aqueous solutions of anionic detergents were found to increase the permeability of water through human epidermis (99, 116, 117).

The capacity to damage the skin appears to be a general property of charged surfactants (28, 108). The presence of anionic surfactants within the skin tissues greatly reduces the amount of "bound water" as measured by desorption experiments (43, 52). The differences between anionic surfactants in their effects on water binding may be explained in terms of the damage they do to the corneum cell membranes, different degrees of damage resulting in the extraction of different quantities of water-soluble substances (108).

The influence of anionic surfactants may be related to their ability to increase the permeability of the skin to water by altering the physical state of water in the skin in such a way as to permit greater freedom to the passage of charged hydrophilic substances (89). Anionic surfactants apparently bind strongly with the protein and cause a reversible denaturation and an uncoiling of the filaments. This is accompanied by a gross expansion of the tissue. Water diffusion in the presence of the soap is much easier through the expanded and unbound water regions than in the original membrane (28). In actuality, the mechanism of the loss of capacity of corneum to bind water in the presence of surfactants is still not understood (10).

(Received July 31, 1972)

REFERENCES

- (1) Blank, I. H., Further observations on factors which influence the water content of the stratum corneum, *J. Invest. Dermatol.*, **21**, 259 (1953).
- (2) Blank, I. H., Factors which influence the water content of the stratum corneum, *Ibid.*, **18**, 433 (1952).
- (3) Laden, K., Natural moisturizing factors in skin, *Amer. Perfum. Cosmet.*, **82**, 77 (1967).
- (4) Gaul, L. E., Relation of dew point and barometric pressure to horny layer hydration, *Proc. Sci. Sec. Toilet Goods Ass.*, **40**, 1 (1963).
- (5) Gaul, L. E., and Underwood, G. B., Relation of dew point and barometric pressure to chapping of normal skin, *J. Invest. Dermatol.*, **17**, 9 (1951).
- (6) Katz, M., and Paulsen, B. J., Absorption of drugs through the skin, in Brodie, B. B., and Gillette, J., *Handbook of Experimental Pharmacology*, Springer-Verlag, Berlin, 1971, Vol. 28.

- (7) Onken, H. D., and Moyer, C. A., The water barrier in human epidermis, *Arch. Dermatol.*, **87**, 584 (1963).
- (8) Berenson, G. S., and Burch, G. E., Studies of diffusion of water through dead human skin, *Amer. J. Trop. Med.*, **31**, 842 (1951).
- (9) Blank, I. H., Cutaneous barrier, *J. Invest. Dermatol.*, **45**, 249 (1965).
- (10) Scheuplein, R. J., and Blank, I. H., Permeability of the skin, *Physiol. Rev.*, **51**, 702 (1971).
- (11) Baker, H., and Kligman, A. M., Measurement of transepidermal water loss by electrical hygrometry, *Arch. Dermatol.*, **96**, 441 (1967).
- (12) Burch, G. E., and Winsor, T., Rate of insensible perspiration locally through living and dead human skin, *Arch. Intern. Med.*, **74**, 437 (1946).
- (13) Singer, E. J., and Vinson, L. J., The water-binding properties of skin, *Proc. Sci. Sect. Toilet Goods Ass.*, **46**, 29 (1966).
- (14) Mali, J. W. H., The transport of water through the human epidermis, *J. Invest. Dermatol.*, **27**, 451 (1956).
- (15) Matoltsy, A. G., Downes, A. M., and Sweeney, T. M., Studies of the epidermal water barrier. Part 2. Investigation of the chemical nature of the water barrier, *Ibid.*, **50**, 19 (1968).
- (16) Wurster, D. E., and Kramer, S. F., Investigations of some factors influencing percutaneous absorption, *J. Pharm. Sci.*, **50**, 288 (1961).
- (17) Buettner, K., Diffusion of water vapor through small areas of human skin in a normal environment, *J. Appl. Physiol.*, **14**, 269 (1959).
- (18) Burch, G. E., and Winsor, T., Diffusion of water through dead plantar, palmar and dorsal human skin and through toe nails, *Arch. Dermatol.*, **53**, 39 (1944).
- (19) De Long, C. W., Thompson, R. C., and Kornberg, H. A., Percutaneous absorption of tritium oxide, *Amer. J. Roentgenol., Radium Ther. Nucl. Med.*, **71**, 1038 (1954).
- (20) Scheuplein, R. J., Mechanism of percutaneous absorption. I. Routes of penetration and the influence of solubility, *J. Invest. Dermatol.*, **45**, 334 (1965).
- (21) Spruit, D., and Malten, K. E., Epidermal water-barrier formation after stripping of normal skin, *Ibid.*, **45**, 6 (1965).
- (22) Cronin, E., and Stoughton, R. B., Percutaneous absorption. Regional variations and the effect of hydration and epidermal stripping, *Brit. J. Dermatol.*, **74**, 265 (1962).
- (23) Vickers, C. F. H., Existence of a reservoir in the stratum corneum, *Arch. Dermatol.*, **88**, 20 (1963).
- (24) Fox, C., Tassoff, J. A., Rieger, M. M., and Deem, D. E., Modification of the water-holding capacity of callus by pretreatment with additives, *J. Soc. Cosmet. Chem.*, **13**, 263 (1962).
- (25) Wurster, D. E., and Munies, R., Factors influencing percutaneous absorption of methyl ethyl ketone, *J. Pharm. Sci.*, **54**, 554 (1965).
- (26) Idson, B., Biophysical factors in skin penetration, *J. Soc. Cosmet. Chem.*, **22**, 615 (1971).
- (27) Fritsch, W. F., and Stoughton, R. B., The effect of temperature and humidity on the penetration of C¹⁴ acetylsalicylic acid in excised human skin, *J. Invest. Dermatol.*, **41**, 307 (1963).
- (28) Scheuplein, R., and Ross, L., Effects of surfactants and solvents on the permeability of the epidermis, *J. Soc. Cosmet. Chem.*, **21**, 853 (1970).
- (29) McKenzie, A. W., and Stoughton, R. B., Method for comparing percutaneous absorption of steroids, *Arch. Dermatol.*, **86**, 608 (1962).
- (30) Hall-Smith, S. P., "Polythene" coverings in dermatological treatment, *Brit. Med. J.*, **2**, 1233 (1962).
- (31) Sulzberger, M. B., and Witten, V. H., Thin plastic films in topical dermatologic therapy, *Arch. Dermatol.*, **84**, 1027 (1961).
- (32) Witten, V. H., Stein, S. J., and Michaelides, P., Use of thin pliable film in selected dermatoses, *Ibid.*, **87**, 458 (1963).
- (33) Jacobi, O., Hygroscopic properties and the wettability of keratin materials, *Kolloid-Z.*, **114**, 88 (1949).

- (34) Jacobi, O., Water and water vapor absorption of the stratum corneum of the living human skin, *J. Appl. Physiol.*, **12**, 403 (1958).
- (35) Spier, H. W., and Pascher, G., Die Wasserlöslichen Bestandteil der peripheren Hornschicht (Hautoberfläche); Allgemeines, Stickstoffhaltige Substanzen, *Arch. Klin. Exp. Dermatol.*, **199**, 411 (1955).
- (36) Spier, H. W., and Pascher, G., Freie aminosäuren an der hautoberfläche, *Ibid.*, **200**, 59 (1955).
- (37) Spier, H. W., and Pascher, G., Die Wasserlöslichen Bestandteil der peripheren Hornschicht (Hautoberfläche); Stickstofffreie Säuren und Basen, *Ibid.*, **201**, 181 (1955).
- (38) Pascher, G., Die Wasserlöslichen Bestandteile der peripheren Hornschicht (Hautoberfläche); α -pyrrolidincarbonsäure, *Ibid.*, **203**, 234 (1956).
- (39) Pascher, G., and Spier, H. W., Die Wasserlöslichen Bestandteile der peripheren Hornschicht (Hautoberfläche); die Ursache der Thermolabilität des Oberflächen-pH, *Ibid.*, **203**, 239 (1956).
- (40) Pascher, G., von Steinvuck, G., and Spier, H. W., Die Wasserlöslichen Bestandteile der peripheren Hornschicht (Hautoberfläche); zur inhomogenen Verteilung von α -Amino Stickstoff, Milchsäure, Chlorid, Kalium (und Natrium) im Stratum Disjunctum, *Ibid.*, **204**, 140 (1957).
- (41) Szakall, A., Über die Eigenschaften, Herkunft und physiologischen Funktionen der die H-Ionenkonzentration bestimmenden Wirkstoffe in der verhornten Epidermis, *Ibid.*, **201**, 331 (1955).
- (42) Szakall, A., Physiologische Prinzipien bei der Entwicklung wirksamer Präparate zur Gesunderhaltung der Haut, unter besonderer Berücksichtigung der Verhütung von Beruflichen Hautschäden, *Arzneim.-Forsch.*, **7**, 408 (1957).
- (43) Blank, I. H., and Shappirio, E. B., Water content of the stratum corneum. III. Effect of previous contact with aqueous solutions of soaps and detergents, *J. Invest. Dermatol.*, **25**, 391 (1955).
- (44) Middleton, J. D., The mechanism of water binding in stratum corneum, *Brit. J. Dermatol.*, **80**, 437 (1968).
- (45) Park, A. C., and Baddiel, C. B., Rheology of stratum corneum II. A physico-chemical investigation of factors influencing the water content of the corneum, *J. Soc. Cosmet. Chem.*, **23**, 13 (1972).
- (46) Flesch, P., Chemical basis of emollient function in horny layers, *Proc. Sci. Sect. Toilet Goods Ass.*, **40**, 12 (1963).
- (47) Jelenko, C., Purification of the water-holding lipid of intact skin and burn eschar, *Amer. Surg.*, **35**, 864 (1969).
- (48) Laden, K., and Morrow, R., Torsional measurements on skin, *J. Soc. Cosmet. Chem.*, **21**, 417 (1970).
- (49) Laden, K., and Spitzer, R., Identification of a natural moisturizing agent in skin, *Ibid.*, **18**, 351 (1967).
- (50) Kligman, A. M., in Montagna, W., and Lobitz, W. C., Jr., *The Epidermis*, Academic Press, New York, 1964.
- (51) Scheuplein, R. J., Analysis of permeability data for the case of parallel diffusion pathways, *Biophys. J.*, **6**, 1 (1966).
- (52) Scheuplein, R. J., and Morgan, L. J., "Bound-water" in keratin membranes measured by a microbalance technique, *Nature*, **214**, 456 (1967).
- (53) Grice, K. A., and Bettley, F. R., Skin water loss and accidental hypothermia in psoriasis, ichthyosis and erythroderma, *Brit. Med. J.*, **IV**, No. 5573, 195 (1967).
- (54) Parmley, T. H., and Seeds, A. E., Fetal skin permeability to isotopic water in early pregnancy, *Amer. J. Obstet. Gynecol.*, **108**, 128 (1970).
- (55) Scheuplein, R. J., Molecular structure and diffusional processes across intact epidermis, Final Comprehensive Rep. No. 7, Springfield, U.S. Dept. of Commerce, 1966.
- (56) Wang, J. H., Self-diffusion and structure of liquid water. I. Measurements of self-diffusion of liquid water with deuterium as a chaser, *J. Amer. Chem. Soc.*, **73**, 510 (1951).

- (57) Spruit, D., The water-barrier of stripped and normal skin, *Dermatologica*, **141**, 54 (1970).
- (58) Jelenko, C., and Ginsburg, J. M., Water-holding lipid and water transmission through homeothermic and poikilothermic skins, *Proc. Soc. Exp. Biol. Med.*, **136**, 1059 (1971).
- (59) King, G., Sorption of large molecules by keratin, *Trans. Faraday Soc.*, **43**, 552 (1947).
- (60) Bettley, F. R., *The Epidermal Barrier and Percutaneous Absorption*, in Champion, R. H., Gillman, T., Rook, A. J., and Sims, R. T., *Introduction to Biology of Skin*, Blackwell, London, 1970.
- (61) Bettley, F. R., and Grice, K. A., A method for measuring the transepidermal water loss, and a means of inactivating sweat glands, *Brit. J. Dermatol.*, **77**, 627 (1965).
- (62) Pinson, E. A., Evaporation from human skin with sweat glands inactivated, *Amer. J. Physiol.*, **137**, 492 (1942).
- (63) Downes, A. M., Sweeney, T. M., and Matoltsy, A. G., Studies of the epidermal water barrier. I. An improved *in vitro* method for determining the diffusion of water through the skin, *J. Invest. Dermatol.*, **49**, 230 (1967).
- (64) Felsher, Z., and Rothman, S., The insensible perspiration of the skin, *Ibid.*, **6**, 271 (1945).
- (65) Swanbeck, G., A new treatment of ichthyosis and other hyperkeratotic conditions, *Acta Dermato-Venereol.*, **48**, 123 (1968).
- (66) Sternberg, T. H., Levan, P., and Wright, E. T., The hydrating effect of pregnenolone acetate on the human skin, *Curr. Ther. Res.*, **3**, 469 (1961).
- (67) Grant, J. K., The metabolism of steroids by man, *Brit. J. Dermatol.*, **81**, Suppl 2, 18 (1969).
- (68) Dempsey, R. E., DeMarco, J. D., and Marcus, A. D., An *in vitro* study of the relative moisture occlusive properties of several topical vehicles and Saran wrap, *J. Invest. Dermatol.*, **44**, 361 (1965).
- (69) Shelmire, J. B., Factors determining the skin-drug-vehicle relationship, *Arch. Dermatol.*, **82**, 24 (1960).
- (70) Spruit, D., Interference of some substances with water vapor loss of human skin, *Amer. Perfum. Cosmet.*, **86**, 27 (1971).
- (71) Memschel, H., Zur kolloidchemie und pharmakologie der keratinsubstanzen der menschlichen haut, *Arch. Exp. Pathol. Pharmacol.*, **110**, 1 (1925).
- (72) Buettner, K., Diffusion of water and water vapor through human skin, *J. Appl. Physiol.*, **6**, 229 (1953).
- (73) Tregear, R. T., *Physical Functions of Skin*, Academic Press, London, 1966.
- (74) Crank, J., *The Mathematics of Diffusion*, Oxford Univ. Press, London, 1957.
- (75) Grice, K., Sattar, H., and Baker, H., The effect of ambient humidity on transepidermal water loss, *J. Invest. Dermatol.*, **58**, 343 (1972).
- (76) Mole, R. H., The relative humidity of the skin, *J. Physiol.*, **107**, 399 (1948).
- (77) Goodman, A. B., and Wolf, A. V., Insensible water loss from human skin as a function of ambient vapour concentration, *J. Appl. Physiol.*, **26**, 203 (1969).
- (78) Scheuplein, R. J., Mechanism of percutaneous absorption. II. Transient diffusion and the relative importance of various routes of skin penetration, *J. Invest. Dermatol.*, **48**, 79 (1967).
- (79) Blank, I. H., Scheuplein, R. J., and Mac Farlane, D. J., Mechanism of percutaneous absorption. III. The effect of temperature on the transport of non-electrolytes across the skin. *Ibid.*, **49**, 582 (1967).
- (80) Spier, H. W., and Pascher, G., Zur analytischen und funktionellen physiologie der hautoberfläche, *Hautarzt*, **7**, 55 (1956).
- (81) Stüpel, H., and Szakall, A., *Die Wirkung von Waschmitteln auf die Haut*, Hüthig, Heidelberg, 1957.
- (82) Jacobi, O. K., About the mechanism of moisture regulation in the horny layer of the skin, *Proc. Sci. Sect. Toilet Goods Ass.*, **31**, 22 (1959).
- (83) Bolliger, A., and Gross, R., Non-keratins of avian and mammalian skin flakes, *Aust. J. Exp. Biol. Med. Sci.*, **32**, 747 (1954).
- (84) Laden, K., A comparative chemical study of dandruff flakes, skin scrapings and cal-lus, *J. Soc. Cosmet. Chem.*, **16**, 491 (1965).

- (85) Spier, H. W., and Schwarz, E., Chemie der Hornschicht in *Proc. XII Int. Congr. Dermatol., Excerpta Med. Found.*, **1**, 389 (1962).
- (86) Laden, K., and Spitzer, R., Identification of a natural moisturizing agent in skin, *J. Soc. Cosmet. Chem.*, **18**, 351 (1967).
- (87) Jacobi, O. K., Nature of cosmetic films on the skin, *Ibid.*, **18**, 149 (1967).
- (88) Sweeney, T. M., and Downing, D. T., The role of lipids in the epidermal barrier to water diffusion, *J. Invest. Dermatol.*, **55**, 135 (1970).
- (89) Sprott, W. E., Surfactants and percutaneous absorption, *Trans. St. John's Hosp. Dermatol. Soc. (London)*, **51**, 186 (1965).
- (90) Bulgin, J. J., and Vinson, L. J., The use of differential thermal analysis to study the bound water in stratum corneum membranes, *Biochim. Biophys. Acta*, **136**, 551 (1967).
- (91) Crouse, R. G., Epidermal keratin: A reevaluation, *Nature*, **200**, 539 (1963).
- (92) Rothman, S., *Physiology and Biochemistry of the Skin*, The University of Chicago Press, Chicago, 1954, p. 239.
- (93) Matoltsy, A. G., The envelope of epidermal horny cells, *Congr. Inst. Dermatol.*, **13**, 1014 (1967).
- (94) Stoughton, R. B., and Rothman, S., in *The Human Integument*, Publ. No. 54, AAAS, Washington, D.C., 1959.
- (95) Monash, S., and Blank, H., Location and re-formation of the epithelial barrier to water vapor, *AMA Arch. Dermatol.*, **78**, 710 (1958).
- (96) Szakall, A., Hautphysiologie Forschung und gesunderhaltung der Haut, *Fette, Seifen, Anstrichm.*, **53**, 399 (1951).
- (97) Singer, E. J., Wegmann, P. C., Lehman, M. D., Christensen, M. S., and Vinson, L. G., Barrier development, ultrastructure, and sulfhydryl content of the fetal epidermis, *J. Soc. Cosmet. Chem.*, **22**, 119 (1971).
- (98) Loveday, D. E., An *in vitro* method for studying percutaneous absorption, *Ibid.*, **12**, 224 (1961).
- (99) Bettley, F. R., and Donoghue, E., Effect of soap on the diffusion of water through isolated human epidermis, *Nature*, **185**, 17 (1960).
- (100) Treherne, J. E., The permeability of skin to some non-electrolytes, *J. Physiol.*, **133**, 171 (1956).
- (101) Matoltsy, A. G., Schrogger, A., and Matoltsy, M. N., Observations on regeneration of the skin barrier, *J. Invest. Dermatol.*, **38**, 251 (1962).
- (102) Spruit, D., and Malten, K. E., The regeneration rate of the water vapor loss of heavily damaged skin, *Dermatologica*, **132**, 115 (1966).
- (103) Monash, S., Location of the superficial barrier to skin penetration, *J. Invest. Dermatol.*, **29**, 367 (1957).
- (104) Blank, I. H., and Scheuplein, R. J., Transport into and within the skin, *Brit. J. Dermatol.*, **81**, Suppl. 4, 4 (1969).
- (105) Burch, G. E., and DePasquale, N. P., *Hot Climates, Man and his Heart*, Thomas, Springfield, Ill., 1962.
- (106) Kligman, A. M., The use of sebum, *Brit. J. Dermatol.*, **75**, 307 (1963).
- (107) Tregear, R. T., *The Permeability of the Skin to Molecules of Widely Differing Properties*, in Rook, A., and Champion, R. H., *Progress in the Biological Sciences in Relation to Dermatology*, **2**, University Press, Cambridge, 1964.
- (108) Middleton, J. D., The mechanism of action of surfactants on the water-binding properties of isolated stratum corneum, *J. Soc. Cosmet. Chem.*, **20**, 399 (1969).
- (109) Blank, I. H., Gould, E., and Theobald, A. B., Penetration of cationic surfactants into skin, *J. Invest. Dermatol.*, **42**, 363 (1964).
- (110) Allenby, A. C., Fletcher, J., Schock, C., and Tees, T. F. S., The effect of heat, pH and organic solvents on the electrical impedance and permeability of excised human skin, *Brit. J. Dermatol.*, **81**, Suppl. 4, 31 (1969).
- (111) Mustakallio, K. K., Kiistala, V., Piha, H. J., and Nieminen, E., Lipids extracted by DMSO from full thickness sheets of epidermis, *Scand. J. Clin. Lab. Invest.*, **19**, Suppl. 95, 50 (1967).

- (112) Dugard, P. H., and Embery, G., The influence of dimethyl sulfoxide on the percutaneous migration of potassium butyl (³⁵S) sulphate, potassium methyl (³⁵S) sulfate, *Brit. J. Dermatol.*, **81**, *Suppl. 4*, 69 (1969).
- (113) Rammler, D. H., and Zaffaroni, A., Biological implications of DMSO based on a review of its chemical properties. *Ann. N.Y. Acad. Sci.*, **141**, 13 (1967).
- (114) Sweeney, T. M., Downes, A. M., and Matoltsy, A. G., The effect of dimethyl sulfoxide on the epidermal water barrier, *J. Invest. Dermatol.*, **46**, 300 (1966).
- (115) Bettley, F. R., The influence of detergents on epidermal permeability, *Brit. J. Dermatol.*, **77**, 98 (1965).
- (116) Bettley, F. R., Influence of soap on the permeability of the epidermis, *Ibid.*, **73**, 448 (1961).
- (117) Bettley, F. R., Irritant effect of soap in relation to epidermal permeability, *Ibid.*, **75**, 113 (1963).