FACTORS WHICH INFLUENCE THE WATER CONTENT OF THE STRATUM CORNEUM*

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If a piece of cornified epithelium, such as a cutting from a callus on the plantar surface of the foot, is dried out, it becomes very hard and brittle, in spite of any natural oils it may contain. Any attempt to soften this cornified epithelium with petrolatum, anhydrous lanolin or natural glycerides of fatty acids, such as olive oil, meets with complete failure, even though the oils remain in contact with the cornified epithelium for months at normal or elevated temperatures. If, on the other hand, similar pieces of cornified epithelium are allowed to absorb a little moisture, they become soft and pliable. It is apparent, therefore, that the water content of the cornified epithelium is a more important factor in maintaining the flexibility of this layer than is its oil content. For this reason we have undertaken a study of the factors which influence the moisture content of the stratum corneum.

Under normal conditions the stratum corneum receives moisture from the fluids which bathe the underneath layers of the skin. It also receives moisture from the sweat glands when they are active. Over a major portion of the body, however, the sweat glands are active only at temperatures above 30°C. at which temperature they are called upon to aid in the removal of heat from the body. Pinson (1) observed that the rate of water loss from the skin of a subject at rest at moderate temperatures was the same as the rate of loss from another area of this subject's skin in which area the sweat glands had been inactivated. We shall not be concerned with the moisture which the stratum corneum may receive from the sweat glands.

On the unclothed areas of skin, water will be lost from the stratum corneum by evaporation into the surrounding atmosphere. In this paper we shall discuss the transfer of water across pieces of excised human skin, taken a few hours after death, and the loss of water from the surface of these pieces of skin under various conditions of temperature, relative humidity, and air flow.

Much of the data was obtained by the method described by Burch and Winsor (2) in which a piece of skin, free of subcutaneous fat, fits tightly over the top surface of a hollow aluminum cylinder filled with water (Figure 1). The entire assembly is weighed; the weight ranges from 50 to 55 grams. It is seen that the chamber is so constructed that if it loses weight when the skin is in place, water has diffused through the skin and been lost by evaporation from the surface into the surrounding atmosphere. In our apparatus water is lost from a 3 sq. cm. area of skin.

A. The Major Barrier Against Water Loss is in the Epidermis

When full thickness abdominal skin is placed on this diffusion chamber and the chamber is maintained at a temperature of 23°C. ± 1.0°C. and at a relative humidity of 40 to 50 per cent in quiet air, water is lost at the rate of 0.1 to 0.2 mg./cm.^{2}/hr. (It seems advisable to give many of the results as ranges of values rather than single values because there is some variation from skin to skin and because there is some change in the rate of water loss as the piece of skin remains on the chamber.) If a similar piece of skin is first subjected to a negative pressure so as to produce a bulla at the dermal-epidermal junction by the method

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described by Blank and Miller (3) and the epidermis removed, the resulting dermis will lose water at the rate of 10.0 to 15.0 mg./cm.^{2}/hr. when placed on the diffusion chamber and held under conditions similar to those for the full thickness skin. These values are quite comparable to those found by Burch and Winsor (2).

It is seen that, by the process of diffusion, water can be brought to the base of the epidermis at least 50 to 100 times as fast as it is lost from the surface of the epidermis. Thus, the major barrier against loss of water from the body is in the epidermis.

In an attempt to locate the barrier, Winsor and Burch (4) sandpapered skin lightly and heavily. Light sandpapering increased the rate of water loss somewhat and heavy sandpapering made the rate of water loss about equal to that observed for skin from which the entire epidermis was removed. They concluded

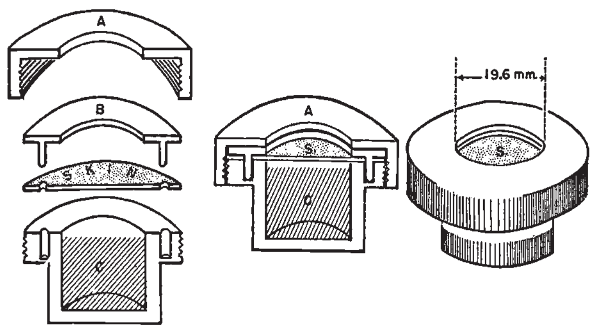

FIG. 1. Diffusion chamber used for the measurement of the diffusion of water through the skin. *A*. Cap. *B*. Gasket with pins. *C*. Vessel for holding the water.

that "the corneum is the layer mainly responsible for the inhibition of diffusion." If the position of the barrier could be determined more exactly and/or more knowledge gained as to the nature of the barrier, we would be aided in our study of the moisture content of the stratum corneum.

B. The Major Barrier Against Water Loss is Not a Lipid Film on the Surface

Wigglesworth (5) studied this problem in insects and found that the barrier was a thin film of hydrophobic lipids which covered the epicuticle. It seems unlikely that in man a barrier exists at the same relative place. The evidence in support of this statement follows.

1. The stratum corneum normally has a relatively low moisture content. The foot of a living male was exposed to the air at room temperature for 2 hours; sweating was at a minimum. Cuttings from a callus on the plantar surface of the foot showed a moisture content of approximately 20 per cent. If the barrier were on the surface of the skin, it would be expected that the moisture content would be nearer that of the underlying tissues (70 to 80 per cent).

2. The stratum corneum easily and quickly takes up water when the skin is immersed in water. It is unlikely that there would be a barrier on the surface

of the skin which would allow the entrance of water into the stratum corneum from the outside but not permit the loss of water to the outside.

3. When an artificial barrier, such as adhesive plaster or petrolatum, is held on the intact, living, non-sweating skin for some time, the stratum corneum becomes macerated (hydrated). If there were a natural barrier on the surface, similar to the applied barrier, then the stratum corneum would always have the characteristics normally associated with maceration.

4. The stratum corneum of a piece of heel skin was artificially hydrated by holding it in distilled water at 23°C. for 2½ hours, subsequently blotted to remove the excess surface water and then placed on the diffusion chamber with the epidermal side out. The rate of water loss at 23°C. and 50 per cent relative humidity was observed by taking a weight of the chamber every five minutes

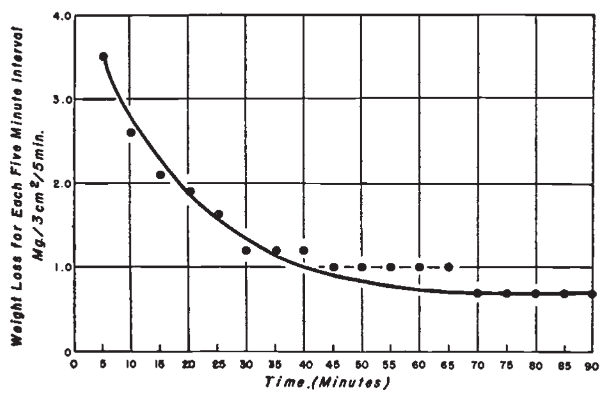

FIG. 2. Showing the rate of water loss from plantar skin at 23°C. and 50% relative humidity subsequent to complete immersion of the skin in water for 2.5 hours.

over a period of 90 minutes (Figure 2). The rate of loss continuously decreases to the constant value at which water is lost from within the skin. The excess which was lost during the first few minutes over the constant value represents water being lost from the hydrated epidermis and indicates that when there is excess water in the epidermis it can be lost at a faster rate than it is received from the underlying tissues. This would not occur if the limiting barrier were on the very surface of the skin.

5. Winsor and Burch (4) showed that washing the surface of the skin with fat solvents did not increase the rate of water loss. We repeated this experiment and obtained similar results. This would indicate that there is no lipid layer on the surface of the skin which serves as the barrier against water loss. If, however, a piece of whole skin from the abdominal area is thoroughly extracted by allowing it to remain in a large volume of a mixture of 8 per cent ethyl alcohol and 92 per cent ethyl ether at room temperature for five days, and if it is subsequently allowed to hydrate in water and then placed on the diffusion chamber, it loses water at a rate 20 to 50 times as fast as normal unextracted skin.

From the above observations it can be concluded that the barrier which prevents the rapid loss of water through the skin is not on the surface of the skin and that under some environmental conditions at least, the stratum corneum tends to dry out. It may be questioned, however, whether the stratum corneum will dry out under all conditions of temperature, relative humidity, and air flow. If there are some environmental conditions under which water will evaporate from the surface of the skin even more slowly than the slow rate at which it reaches the stratum corneum from the underlying tissues and diffuses through it, then water will tend to accumulate in the stratum corneum and it will not dry out.

C. Under a Wide Range of Environmental Conditions, Water is Lost from the Surface More Rapidly than it Reaches the Surface

The effect of environmental conditions on the rate of evaporation alone can be determined by measuring the rate of water loss from the surface of water under varying conditions. We have not been able to devise a method for measuring the rate at which water is brought to the stratum corneum itself from the underlying tissues, but we can study the rate at which it diffuses through the dermis and subsequently evaporates from this surface under the conditions of the experiment. Thirdly, the effect of environmental conditions on the rate of water loss from full thickness skin can be observed. From this type of data we can learn at what rate water is brought to the base of the epidermis and assume that this may not be too different from the rate at which it is brought to the base of the stratum corneum. We also learn the rate at which water is lost from the surface of the stratum corneum and can compare this loss to that which occurs from the surface of water under similar environmental conditions.

A piece of full thickness abdominal skin and an adjacent piece from which the epidermis had been removed by negative pressure were placed on diffusion chambers. A third chamber contained water but was not covered with skin. These chambers were held in desiccators (22 cm. in diameter and 16 cm. deep) on top of a screen in an inverted position (except the chamber containing water alone). In the bottom of the desiccator were 500 ml. of sulfuric acid of 1.296 specific gravity which maintained a relative humidity of 57 per cent at 23°C. (6). The loss was measured for a two-hour period. The chambers were then transferred to a similar desiccator containing a sulfuric acid solution which maintained the same relative humidity at 35°C. Again the water loss was measured for a two-hour period.

A similar experiment was run where the water loss was measured at two relative humidities but at the same temperature (23°C.). The first desiccator contained sulfuric acid of 1.137 specific gravity and maintained a relative humidity of 88 per cent; in the second the specific gravity was 1.473 with a relative humidity of 23 per cent.

In a third experiment the water loss was measured at 23°C. and a relative humidity of 25 to 35 per cent first in quiet air and secondly in rapidly flowing air from an electric fan.

The results of these experiments are summarized in Table I. It is seen that under the conditions studied, water readily diffuses through the dermis and reaches the epidermis in adequate supply. It is likely also that it readily diffuses through the prickle-cell layer and reaches the base of the stratum corneum in adequate supply. However, under none of the conditions studied does water reach the surface of the skin in sufficient amounts to keep pace with the rate of evaporation which could take place. This is seen from the fact that the rate of evaporation from the full thickness skin is far less than the rate of evaporation from a water surface under identical conditions. Under all of these conditions, therefore, it is the barrier and not the environmental conditions which determines the amount of water which will be lost from the surface. The stratum corneum will tend to dry out since more water can be evaporated from the surface than will reach there by diffusion.

D. Relative Humidity is an Important Factor in Determining the Water Content of the Stratum Corneum

However, there is another factor which must be considered before the extent of this drying out can be determined. The textile chemists have for some years

TABLE I

The rate of water loss (mg./cm.²/hr.) from water, dermis and whole skin at various temperatures, relative humidities, and air conditions

TEMPERATURE (°C.)	RELATIVE HUMDITIY (PER CENT)	AIR CONDITION	WATER LOSS FROM		
			Water	Dermis	Whole skin
23	57	Still	15.0	11.3–12.2	0.10–0.20
35	57	Still	27.5	17.6–19.5	0.40–0.80
23	88	Still	5.3	2.7– 4.1	0.07–0.10
23	23	Still	21.8	7.2–10.4	0.13–0.27
23	25–35*	Still†	24.2	7.5–10.2	0.20–0.25
23	25–35*	Flowing	241.4	49.3–67.8	0.25–0.50

* These relative humidities were calculated from wet and dry bulb temperatures in flowing air.

† The diffusion chamber for this test was in an air-conditioned room with no fan blowing on it, but not inside a desiccator.

known that the moisture content of wool is a function of the relative humidity of its environment (7). Wool does not dry out entirely unless held in completely dry air and the higher the relative humidity of the air, the higher will be the moisture content of wool which is in equilibrium with that air. We have made similar observations for cornified epithelium.

A small hook of copper wire was cemented to the under side of the top of a glass weighing bottle, 4 cm. in diameter and 6 cm. deep, and the dry weight of the bottle obtained. On the hook was hung a piece of cornified epithelium obtained from a callus and which was about 2 sq. cm. in area and 0.33 mm. thick. The tops of the weighing bottles are ground so as to be interchangeable and the top with the skin hanging on it was transferred to a second weighing bottle containing 20 ml. of concentrated sulfuric acid and held at 23°C. It was always transferred back to the first bottle for weighing. When a constant weight was reached, the dry weight of the cornified epithelium was obtained. For the piece of epithelium mentioned, the dry weight was 62.7 mg. The concentrated sulfuric acid was then replaced by a diluted sulfuric acid of 1.473 specific gravity which maintained a constant relative humidity of 23 per cent in the weighing bottle. The cornified epithelium was then held in this environment until a constant weight was obtained. Usually about 48 to 72 hours were required. The gain in weight divided by the dry weight multiplied by 100 is the milligrams of water which will be held by 100 milligrams of dry stratum corneum at equilibrium conditions. With increasingly dilute sulfuric acid solutions (increasing relative humidities) the amount of water which will be taken up by cornified epithelium when it is in equilibrium with an atmosphere of any relative humidity can be determined. A curve of such equilibrium conditions is shown in Figure 3.

The cornified epithelium becomes soft and pliable when a moisture content of about 10 mg. per 100 mg. dry weight is reached; this corresponds to a relative humidity of about 60 per cent. Mole (8) used the term "relative humidity of the skin" which he defined as a "measure of the water content of the surface layer of the skin, expressed as a percentage of the maximum when the skin surface is covered with a homogeneous film of water." Mole used this factor in correlating evaporative loss of water from the skin and skin temperature to the relative humidity of the environment.

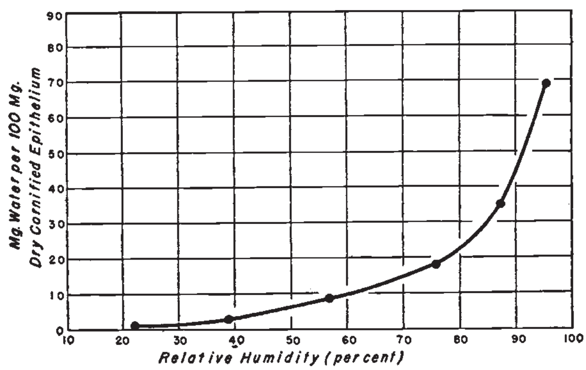

FIG. 3. Showing the relationship between the water content of cornified epithelium and the relative humidity of the environment at 23°C.

Thus we see that the stratum corneum may receive water from the environment (when transferred from a lower to higher humidity) as well as lose water to the environment. It is our opinion that this may be a more important mechanism for the control of the water content of the stratum corneum than is the variation in the amount of water which the stratum corneum receives by diffusion from the underlying tissues.

The conditions at which the stratum corneum will dry out are therefore defined. It will dry out most rapidly at high temperatures, low humidities, and in flowing air. However, it probably cannot be dried out to the point of "brittleness" at any temperature or at any rate of air flow if the relative humidity is maintained at about 60 per cent or more. When it does dry out due to low humidity or rapidly flowing air, it probably becomes brittle enough to break on flexing or stretching. This would explain the fissures and scaling of chapping and windburn.

DISCUSSION AND SUMMARY

The stratum corneum receives water from the sweat glands and from the underlying tissues by diffusion. It loses water to the environment by evaporation.

Low temperature, high relative humidity, and no flow of air across the skin reduce the rate of evaporation from the skin but even under the conditions at which the rate of evaporation is the lowest, water can still be lost at a much more rapid rate than it reaches the surface. The water is kept from reaching the surface by a barrier at some point in the epidermis. This barrier is not a film of hydrophobic lipids on the surface of the skin. If the barrier is not on the surface and if water tends to be lost from the surface at a more rapid rate than it reaches there, the stratum corneum or at least the upper part of it will tend to dry out.

Under most environmental conditions in temperate climates, the stratum corneum would dry out and become brittle were it not for the fact that for relative humidities of about 60 per cent and higher, an equilibrium state exists which does not permit the moisture content to drop below 10 mg. per 100 mg. dry weight. At this moisture content the stratum corneum remains soft and pliable.

Under the low relative humidity of winter weather and heated houses or the rapidly flowing air of windy conditions, the stratum corneum does dry out and the fissures of a chapped skin and scaling of a windburn can develop because of the brittleness of the dried stratum corneum.

The slow rate at which water reaches the surface could be explained by the presence of a very thin barrier at or near the base of the stratum corneum or by a very slow uniform rate of diffusion through the entire stratum corneum. These investigations have not shown specifically which of these two states exists.

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DISCUSSION

Dr. Sulzberger, *New York City:* Obviously this is the beginning of a series of studies of a very important phenomenon. I do not know how many of you are in practice, but if you are in dermatologic practice, I am sure you hear every day—"what makes my skin so dry?" "what makes my skin chap so easily?"

Many of the questioners are ladies who have reached an age at which their complexion is beginning to be of great concern to them. But that occurs not only in females of all ages but also in males and in children. And I think that in this realm which the studies of Dr. Blank are beginning to investigate, there lie some answers to extremely important questions having to do with a great variety of different kinds of skin complaints and skin diseases—for example varying from icthyosis, eczemas, chapping and winter itch all the way to the ordinary drying-out of ageing.

I also think that in this complex which he is beginning to study with careful methods of mensuration, there lies something which has to do with the problems of sweat gland occlusion. It appears to me that one cannot disregard entirely the fluid which is being poured on the skin surface by the sweat glands. And Dr. Blank mentioned that he was simply disregarding it for the moment in these studies. I am quite sure that the imbibition of the stratum corneum with fluid, can lead to a swelling and that swelling in turn can easily lead to an impairment of the patency of the surface openings of the ducts, with everything that that happening may carry with it. And I think it may well be the counter-acting of that mechanism by placing patients in temperatures which are relatively high combined with consistent relatively low humidities, it is the counteracting of that mechanism of horny imbibition which may lead to improvement in so many itching skin diseases when they are transferred to relatively dry environments.

Dr. Blank: I don't think there is much to do except to thank Dr. Sulzberger for his additional comment.

It is certainly true that, before we study the whole realm of moisture content of stratum corneum, we must take into consideration the moisture which arrives on the surface from the sweat glands. It is true, of course, that over a major portion of the body sweat glands are not active at temperatures we have been studying, with the possible exception of the few measurements at 35°C. Moisture from the sweat glands is a different problem but must come into the picture sooner or later.