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# Skin Moisturizers. I. Methods for Measuring Water Regain, Mechanical Properties, and Transepidermal Moisture Loss of Stratum Corneum

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**Synopsis**—Four parameters for assessing the interaction between human STRATUM CORNEUM and WATER are described. Two methodologies, ELASTIC MODULUS and STRESS RELAXATION, for determining the MECHANICAL PROPERTIES of stratum corneum have been utilized. It is shown that both of these parameters depend on the moisture content of the stratum corneum, i.e., the ambient relative humidity. The rate of WATER VAPOR ABSORPTION by human stratum corneum, the third parameter examined, is a function of the ambient relative humidity. Surprisingly, the equilibrium moisture content of stratum corneum at humidities below approximately 80% appears to be essentially the same for unextracted stratum corneum and for stratum corneum extracted with lipid solvents. The fourth parameter, the rate of WATER VAPOR TRANSMISSION through stratum corneum *in vitro*, is a linear function of the ambient relative humidity and has been shown to be markedly affected by changes in temperature.

## Introduction

Since Blank's observation in 1952 (1), that the water content of skin is responsible for its softness, the properties of epidermis as a function of its moisture content and of the presence of "natural" and extraneous moisturizers have been studied extensively by cosmetic chemists and dermatologists.

The subject of transepidermal moisture loss *in vivo* under various ambient conditions of humidity, temperature, and air flow has been studied by several investigators. Of particular interest is a paper by Grice *et al.*, who indicated that the relationship between transepidermal water loss and ambient relative humidity *in vivo* is not linear (2). This is not in accord with the data by Wildnauer *et al.*, who suggested that the relationship between mechanical properties and relative humidity is linear (3).

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The relationship between the mechanical properties of stratum corneum and its moisture content has been studied by several techniques (4–6). Much of this work has been conducted on stratum corneum obtained from guinea pig foot pads or human callus. It was pointed out by Kligman that the stratum corneum of guinea pigs allows water to pass three times as fast as human abdominal stratum corneum (7). He also states that "the specialization of the horny layer of the palms and soles is so unique as to require separate status." These tissues contain lower quantities of the water-soluble substances present in abdominal skin and are more permeable to water; data from callus will not "accurately apply to the membranous horny layer." A considerable body of work on the mechanical properties of human skin *in vivo* is not pertinent because it deals primarily with the mechanical properties of the dermis which presumably is not touched by normal cosmetic treatments. Water vapor transmission experiments *in vivo* are made difficult by sweating unless this is repressed by vasoconstricting drugs. As a result, well-controlled experimentation *in vivo* is very difficult.

Substrate variability and the noted complications of *in vivo* studies make it attractive to study mechanical and water-holding properties of human stratum corneum *in vitro*. This study has been designed to study and to compare techniques suitable for measuring the interaction of stratum corneum with water. Part II of this work deals with the effect of a variety of typical cosmetic ingredients in order to establish a rationale for their use in skin treatment preparations.

## Theoretical Treatments and Experimental Procedures

### *Mechanical Properties*

#### *Elastic Modulus*

When a viscoelastic strip of material of length $l$ and cross sectional area $A$ is subjected to a normal tensile force ($f$), the stress ($\sigma$) is $\frac{f}{A}$, and the strain ($\epsilon$) is the fractional increase in length, $\Delta l/l$. Below the yield point, Young's modulus of elasticity ($E$) is the ratio of $\sigma/\epsilon$, i.e., the slope of the load-elongation curve. Since $A$ of the stratum corneum is not constant and is very difficult to measure at all points, it was decided to use the parameter $AxE$ (eq 1) and assume that strips taken from the same specimen of stratum corneum have approximately the same cross-sectional area. It was, therefore, necessary to determine $AxE$ of an untreated control for each specimen of stratum corneum in order to compare the effect of a given treatment.

$$A \times E = \frac{fl}{\Delta l} \tag{1}$$

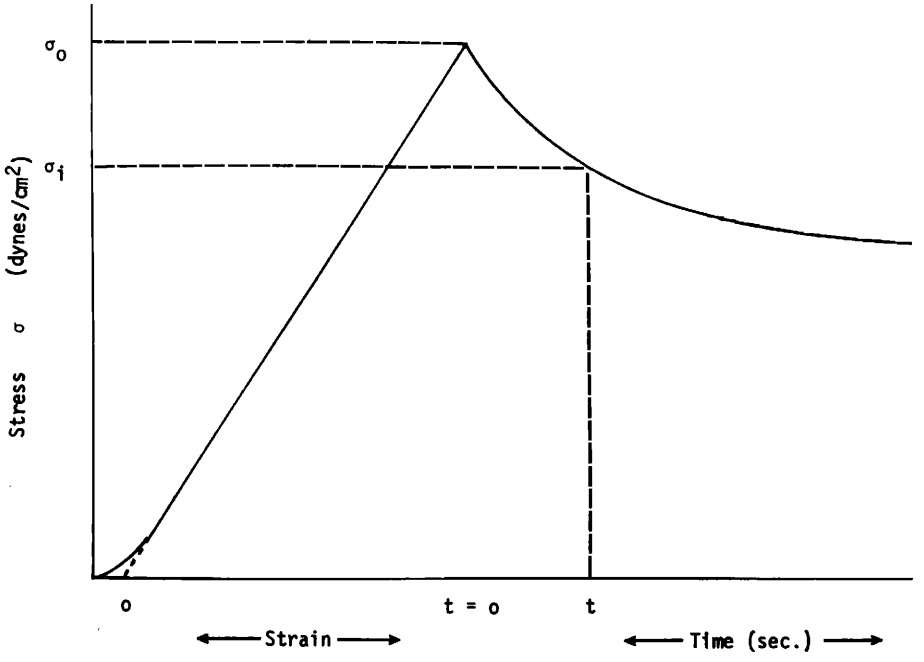


*Figure 1.* Typical loading and relaxation curve for stratum corneum

*Relaxation Function*

When an elastic material is strained (to strain $\epsilon$, Fig. 1) by stressing (to load $\sigma_0$), the value of $\sigma$ will decay as a function of time ($t$) if the strain $\epsilon$ is kept constant. The relaxation modulus $E_r(t)$ at any time $t$ can then be computed by dividing the stress ($\sigma_i$) at time $t_i$ by the strain $\epsilon$ at $t = 0$ (Fig. 1). At $t = 0$, $E_r(t)$ equals Young's modulus as long as the material has not been strained beyond its yield point. Wall *et al.* used the stress relaxation spectrum, $H(\ln\tau)$, to describe the "multiple mechanical relaxation phenomena" in human hair (8). They plotted the derivative of the relaxation modulus with respect to the logarithm of time:

$$H(\ln\tau) = \frac{d(E_r(t))}{d\ln\tau} \tag{2}$$

Since the primary interest here is the effect of moisture on the behavior of stratum corneum, the cross-sectional area was included in the relaxation function. Since a plot of log $AxE_r(t)$ *vs.* log $t$ is essentially linear between 1 and $10^4$ sec, the slope of this line is the only value required to describe the effect of a given moisture condition or cosmetic treatment on the stratum corneum.

The linear behavior described above is typical of amorphous polymers of high molecular weight below their glass transition temperatures (9). The

response to the external stress consists primarily of local adjustments since the chain backbone configuration is essentially immobilized.

*Technique*

A model TM Instron Tensile Tester®* was used for measuring both elastic moduli and relaxation spectra. The rate of travel of the cross head was 2.54 mm/min, which was equivalent to an extension of about 10–15%/min. (The sample length varied between 17 and 25 mm.)

Strips of stratum corneum, 0.5 cm wide by approximately 3.5 cm long, were attached to Bakelite®† tabs with a commercial epoxy cement or Duco®‡ cement. The length of each strip (between tabs) was measured to the nearest 0.01 mm with a cathetometer.§ The samples were stored at the desired relative humidity (RH) for at least 24 hours and then suspended in a cylinder (diameter, 7 cm; length, 15 cm) mounted on the Instron. Air at the same RH was passed through the chamber for 20 min at which time the samples were extended to a load of 4.0 g.

Some obvious minor modifications of the above procedure were required for those stratum corneum strips which were extended under deionized water or test solutions.

*Water Absorption*

A Cahn RG Electrobalance®// was used to measure moisture absorption by stratum corneum or of cosmetic materials applied to samples of stratum corneum or to glass filter paper. The Electrobalance was fitted with an *X-Y* recorder¶ which has an input range of 0.1 mg/2.54 cm, while the graph can be read to 0.002 mg. Equilibrium weights can be estimated to 0.001 mg using a null system. The balance was also fitted with a cube-shaped plastic chamber (7.5 cm on each side) into which air of controlled humidity was blown at a rate of 1200 ml/min. The determination of equilibrium water content was made immediately after stopping the air flow so that air currents would not affect the reading. Equilibration occurs quite rapidly due to the moving air stream (absence of an unstirred layer and the small size of the sample). Attainment of equilibrium was evidenced by constancy of weight for 4 to 5 hours.

*Instron Corp., Canton, Mass.
†Union Carbide Corp., New York, N.Y.
‡E. I. du Pont de Nemours & Co., Wilmington, Del.
§Ealing Corp., Cambridge, Mass.
//Cahn Instrument Co., Paramount, Calif.
¶Model 7035 B with internal time base generator, Hewlett-Packard Co., Palo Alto, Calif.

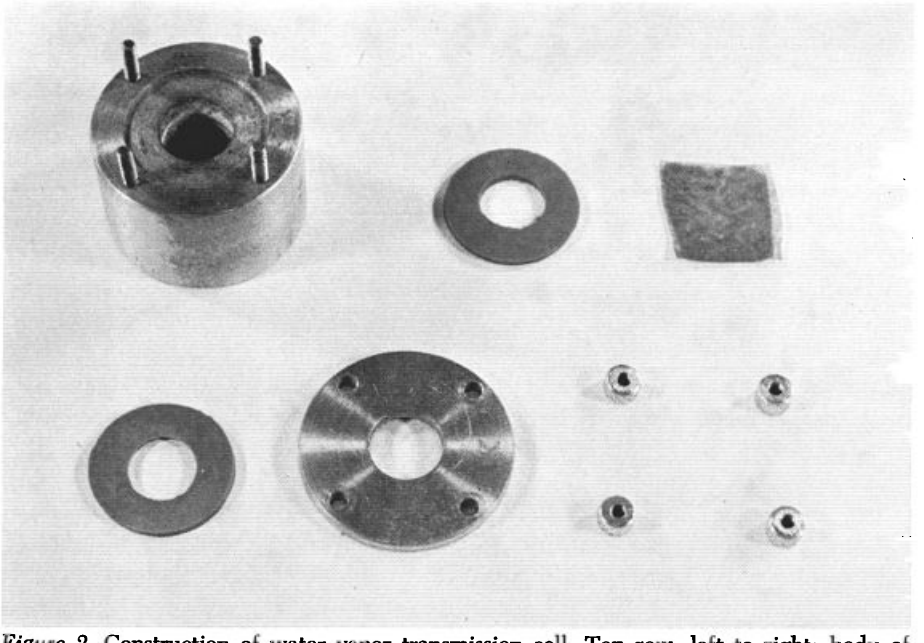

*Figure 2.* Construction of water vapor transmission cell. Top row, left to right: body of cell, lower silicone gasket, stratum corneum. Bottom row, left to right: upper silicone gasket, top plate, closure nuts)

### *Water Vapor Transmission*

Cells for measuring the rate of diffusion of water vapor through stratum corneum were constructed of cylindrical aluminum stock (3.7 cm in diameter x 2.2 cm high) into which was drilled a 1.5-cm deep hole having a diameter of 1.3 cm (Fig. 2). This depression has a capacity of approximately 2 ml. An aluminum top provided with a 1.3-cm hole could be attached to the lower chamber with four bolts. The stratum corneum was placed between two silicone rubber gaskets which were then placed between the chamber and the top. The bolts were attached "finger tight" in order not to deform the silicone gasket. After mounting the stratum corneum on a cell containing 0.3 ml of water, the cell was placed into a chamber containing a constant RH solution or exposed to a stream of humidified air. The cell was weighed every 24 hours until the rate of water loss became constant.

Initial rates of transepidermal water loss are fairly high, and up to 4 days may be required to reach a steady rate. Only those rates were utilized in this study which remained constant for 2 to 3 days after the initial equilibration. Whenever long periods of time were required for testing at several humidities, the sample was examined for signs of mold growth or stretching of the epidermis due to the "vacuum" formed inside the cell as water leaves the cell. The loss of water was computed as mg/cm$^2$/hr.

### *Humidity Control*

In order to obtain dry air (0% RH), the air was first passed through reagent grade sulfuric acid and then magnesium perchlorate* since it has been reported that calcium chloride will permit passage of up to 160 ppm of water in a gas stream (10). In experiments in which constant humidity solutions were used, the saturated solutions of several salts were employed (11).

When an air stream of constant humidity was required for several days, the use of saturated salt solutions is inconvenient due to blockage of the gas dispersion tube by crystallization. Therefore, the apparatus described by Smith (12) was used. This apparatus consists of a proportioning valve and saturators to mix controlled quantities of wet and dry air to obtain a given humidity. The equipment is capable of accurately supplying air at any RH between 0 and 100% in 5% RH steps over a wide range of temperatures.

### *Materials*

Unless otherwise indicated, C.P. reagents were employed throughout. Postmortem abdominal skin was immediately frozen in dry ice. Within 72 hours, the samples were heated in a water bath to 52° ± 2°C, and the stratum corneum was peeled from the dermis as described by Kligman and Christophers (13). The separated stratum corneum was washed in several changes of distilled water and gently picked up on a piece of stainless steel wire mesh. The stratum corneum was air-dried, removed from the screen, and stored over magnesium perchlorate. When extracted stratum corneum was required, it was extracted according to the procedure of Blank (14), i.e., 24 hours in pyridine at room temperature followed by 1-hour extraction with water at room temperature.

## Results and Discussion

### *Mechanical Properties*

In order to avoid experimental artifacts and to conserve material it was decided to determine whether the stratum corneum is elastically isotropic or anisotropic like the dermis. The values of the quantity $AxE$ for stratum corneum extended under water at room temperature were $1.79 \pm 0.12 \times 10^4$ dynes and $1.90 \pm 0.18 \times 10^4$ dynes, respectively, for strips (8 in each direction) cut at right angles to each other. The results indicate that the stratum corneum may be considered isotropic. Adjacent strips of stratum corneum usually have $AxE$ values within ± 5% but occasionally vary by as much as 30–40%

As expected, the elastic modulus of human stratum corneum was found to be a function of the ambient RH. The results of Fig. 3 confirm those of many other investigators and show that stratum corneum becomes "softer" when

*Anhydrone®, J. T. Baker Chemical Co., Philadelphia, N.J.

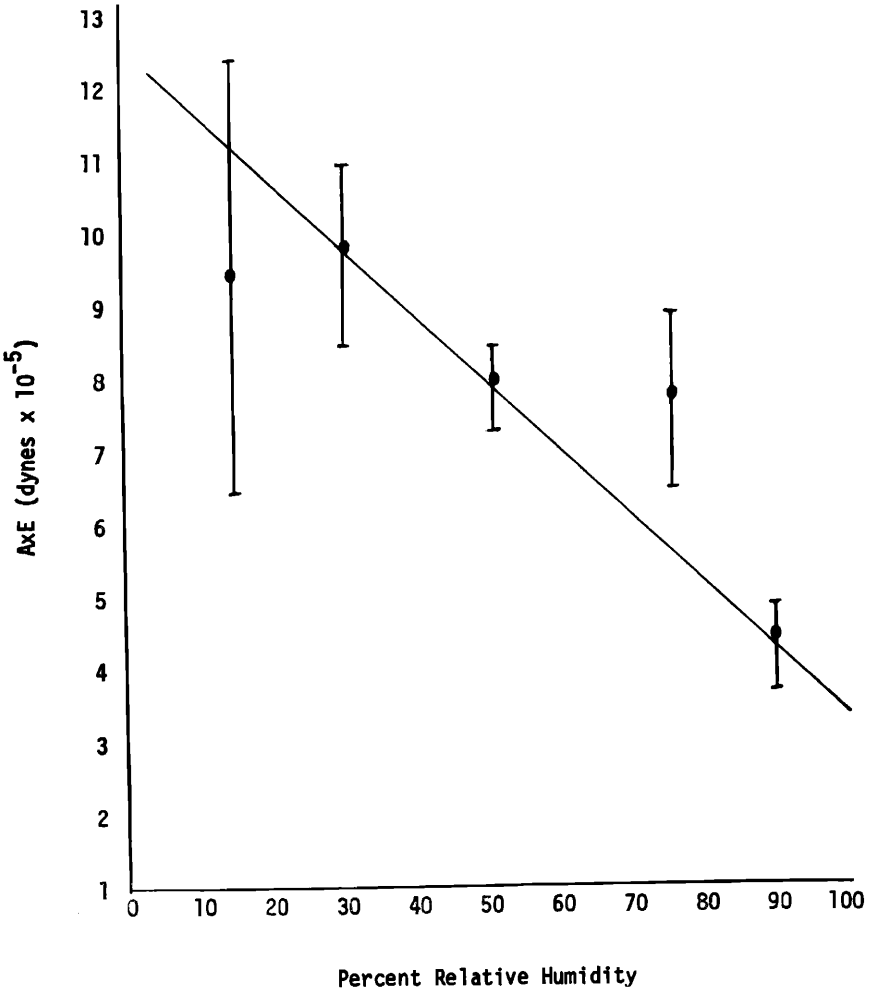


*Figure 3.* Elastic modulus of stratum corneum as a function of relative humidity (vertical bars refer to average deviation)

the RH increases. The average deviations noted in these experiments are quite high. In fact, the large deviation at 15% RH suggests that relatively dry stratum corneum cannot respond as a homogeneous material. Goodman and Wolf (15) also have noted large standard deviations of *in vivo* water transmission data at low RH's. At higher RH's, the higher moisture content tends to make the stratum corneum act in a more homogeneous way, thus lowering the average deviation. The relationship between modulus and RH has been presented in linear form, although Parks and Baddiel (16) have indicated that this relationship is nonlinear above 60% RH. It is noted in passing that different specimens of stratum corneum yield "lines" having different slopes.

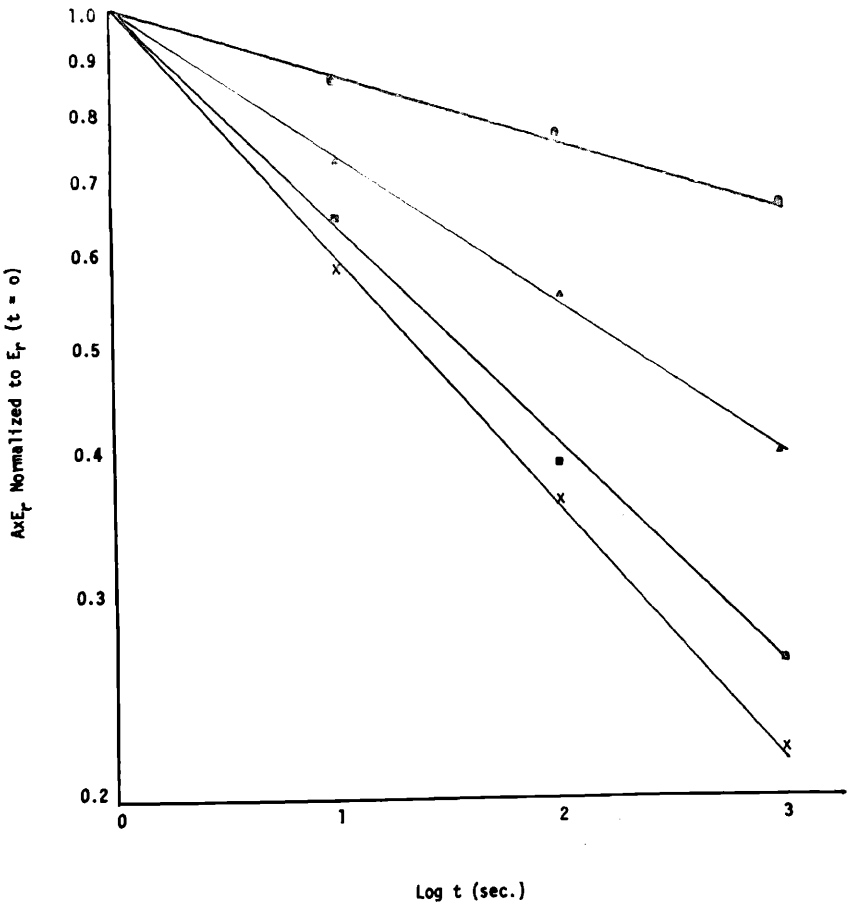


*Figure 4.* Stress relaxation of stratum corneum (log plot of $AxE_r$ *vs.* $t$). Values of $AxE_r$ at any time are divided by $AxE_r$ at time zero

● = 0% RH ■ = 76% RH
◗ = 31% RH X = 100% RH

The stress relaxation experiments were conducted on samples of stratum corneum in humidified air. In preliminary experiments, it was found that for strains between 0.01 and 0.10 the relaxation spectra were essentially identical. Therefore, the samples were stressed to a constant stress (4.0 g) rather than a constant strain, since stress is instrumentally more easily controlled.

At the start it was necessary to demonstrate that the slopes of the lines resulting from a plot of log $AxE_r$ *vs.* log $t$ depend on the humidity of the air. Figure 4 shows that the plots are linear and that their slopes are functions of humidity. It is possible to estimate the errors of the slopes by comparing the slopes resulting from stress relaxation experiments on adjacent pieces of stratum corneum at the same RH. These errors are compared to those obtained by

Table I
Average Deviation of Mechanical Measurements

| % RH | $A \times E$ | | Slope $[H(\ln\tau)]$ | |
|---|---|---|---|---|
| | Value | Av. Dev. | Value | Av. Dev. |
| 100 (4)[a] | 215 | ± 35 | −0.173 | ±0.039 |
| 76 (4) | 280 | ± 29 | −0.164 | ±0.031 |
| 31 (5) | 393 | ±100 | −0.0938 | ±0.004 |
| 0 (4) | 534 | ±121 | −0.0528 | ±0.002 |

[a]Figure in parentheses refers to number of samples tested at each RH.

merely determining the value of $AxE$ for adjacent pieces of stratum corneum at the same RH (Fig. 3). This comparison, shown in Table I, indicates that $AxE$ values are most precise at high RH, while the slope of the stress relaxation function is most precise at low RH's. Accordingly, the stress relaxation function is the preferred measurement under relatively dry conditions.

### *Water Absorption*

The rate of water absorption by strips of unextracted stratum corneum dried over concentrated $H_2SO_4$ and then exposed to different RH's is shown in Fig. 5. In agreement with other investigators, the *rate* of regain was found to be dependent on RH and on the presence of "natural moisturizers." In the examples studied here at high RH (93%), the difference in absorption rates up to about 30 min between the extracted and unextracted stratum corneum is unexpectedly low. Most investigators who have studied the differences in moisture absorption of extracted and unextracted "keratinized" tissue record *equilibrium* moisture absorption. Singer and Vinson (17) record equilibrium moisture contents (at 80% RH) for normal neonatal rat corneum and callus of about 50% and of about 20–30% after solvent + $H_2O$ extraction. The equilibrium moisture contents at RH's up to 80% obtained in this study of unextracted human stratum corneum are much lower and are only marginally dependent on extraction (with pyridine and water). The results are recorded in Table II and were so unexpected that the problem was studied on three specimens of stratum corneum. In all cases, the moisture absorption was determined on a small piece of stratum corneum and was then redetermined on the identical piece after extraction. The weight loss due to extraction generally ranged between 25 and 30%. It is not possible to offer a definitive explanation for the discrepancy of the data obtained here and those reported by other investigators (14). It is noted that the data of Fox *et al.* (18) are close to those found here, and that the significant differences between extracted and unextracted cornified epithelial tissue observed by other investigators occur at RH's > 80%. Such high humidities were usually avoided here because it was difficult to maintain high humidities for the long periods required for equilibration (up to 8–10 hours) at ambient temperature without condensation.

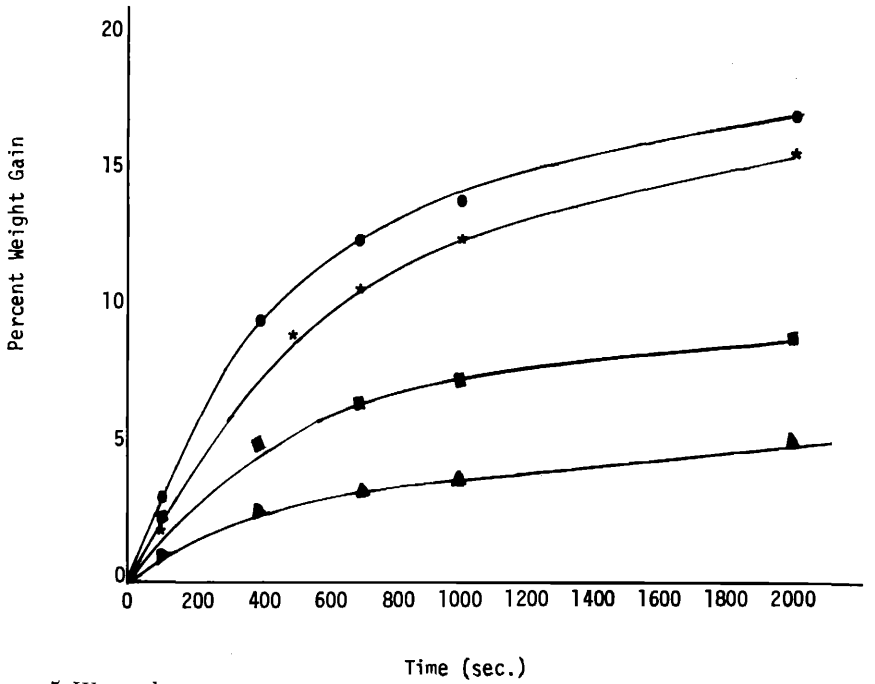


*Figure 5.* Water absorption of stratum corneum at several RH's as a function of time

—●—●—●— 93% RH unextracted
—*—*—*— 93% RH extracted
—■—■—■— 60% RH unextracted
—▶—▶—▶— 30% RH extracted

Table II
Equilibrium Moisture Content of Human Stratum Corneum

| Sample | % RH | % Water Absorbed by Dry Stratum Corneum[a] | |
|---|---|---|---|
| | | Unextracted | Extracted |
| A | 31 | 5.4[b] (4.9-6.0) | 5.3[c] (3.9-6.2) |
| A | 76 | 11.4[b] (10.8-12.1) | 13.3[c] (12.5-14.1) |
| B | 31 | 9.3[c] (8.5-10.3) | 6.5[d] (5.8-7.3) |
| B | 76 | 13.1[d] (12.0-14.2) | 14.1[d] (12.8-15.0) |
| C | 5 | 2.5 | 2.5 |
| C | 15 | 4.0 | **5.0** |
| C | 31 | 4.0 | **5.1** |
| C | 35 | 6.5 | 7.0 |
| C | 50 | 8.6[c] (7.9-9.3) | 9.9[c] (9.0-11.0) |
| C | 75 | 14.5[c] (13.8-15.1) | 15.8[c] (14.2-17.4) |
| C | 90 | 21.9[c] (21.8-22.1) | **22.5**[c] (20.4-24.5) |

[a]Figures in parentheses are ranges.
[b]Average of 5 determinations.
[c]Average of 3 determinations.
[d]Average of 4 determinations.
[e]Average of 2 determinations.

*Water Vapor Transmission*

The results of *in vitro* transepidermal water losses through untreated stratum corneum at 21 ± 1.5°C against a dry atmosphere ($CaCl_2$) show wide variations depending on the stratum corneum specimen. When water vapor is in contact with the stratum corneum inside the cell, the average rate is about 0.25 mg $cm^{-2}$ $hr^{-1}$. When water contacts the stratum corneum, the average transepidermal water loss rises to about 0.30 mg $cm^{-2}$ $hr^{-1}$. These values are fairly close to each other and are in accord with normally accepted values of transepidermal moisture loss obtained *in vivo* (19).

Extraction of stratum corneum, first with pyridine and then with water, increases the rate of transepidermal moisture loss from about 0.2 to as much as 1.6 mg $cm^{-2}$ $hr^{-1}$. This is a clear indication that the extractables (primarily lipids) present in the stratum corneum contribute significantly to slowing down the moisture loss from dermal and epidermal tissue by evaporation.

On theoretical grounds and intuitively one would expect that the transepidermal water loss from a cell of the type described here depends on the RH to which the cell is exposed, i.e., the driving force for diffusion is the chemical potential gradient. That this is the case has been shown here because the plot of water vapor transmission rate *vs.* RH (Fig. 6) is linear with a slope of $-1.95 \times 10^3$ mg $cm^{-2}$ $hr^{-1}$/% RH. These results are different from those reported by Grice *et al.* (2), who indicated that a plot of water vapor transmission *vs.* RH yields a curve showing a maximum between 25 and 50% RH (Fig. 6). It was at first thought that this difference might be due to the fact that an unstirred layer in the system used here was responsible for the discrepancy. However, when the results were repeated under a stream of air (1200 ml/min) aimed at the exposed surface of the stratum corneum, the results were essentially unchanged. It appears, therefore, that the results of the *in vitro* experimentation conducted here conform to the *in vivo* data reported by Goodman and Wolf (15), whose results yield linear plots although the slope of their line has a value of $-4.5 \times 10^3$ mg $cm^{-2}$ $hr^{-1}$/% RH.

The effect of temperature on the permeability of stratum corneum or any membrane follows the Arrhenius equation (20). Thus, a plot of the logarithm of permeability *vs.* $\frac{1}{T}$ is linear and has a slope of $-\frac{E_p}{2.303R}$, where $E_p$ is the activation energy of permeation.

The experimentally measured flux, $J$, is related to permeability ($P$) *via* eq 3,

$$J = P \Delta C \tag{3}$$

where $\Delta C$ = the difference in the concentration of the water on the internal side of the membrane and the external side (moles $l^{-1}$).

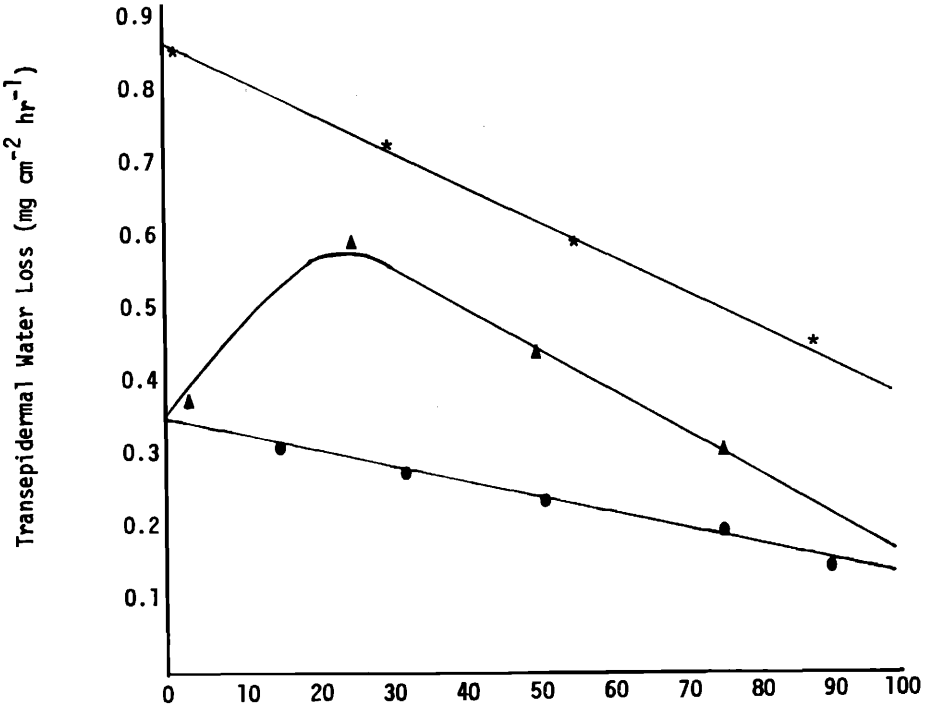


*Figure 6.* Transepidermal water loss vs. relative humidity

—*—*—*— Goodman and Wolf (15)
—▶—▶—▶— Grice *et al.* (2)
—●—●—●— present paper

Thus it is possible to plot log $J$ against $\frac{1}{T}$ (Fig. 7) and compute $E_p$. The value for $E_p$ for fully hydrated stratum codneum cited by Scheuplein and Blank (21) is 13-16 kcal mole$^{-1}$. The clearly expotential temperature dependence of water vapor permeability is proof of transmission by diffusion. Transmission by random kinetic motion through a capillary would be expected to be proportional to $T^{1/2}$ (22). A more comprehensive discussion of the factors involved in diffusion of water through stratum corneum has been given by Berube and Berdick (23).

## Conclusions

1. The mechanical properties of human stratum corneum, as measured by the elastic modulus or by stress relaxation, are dependent on the moisture content of the stratum corneum.

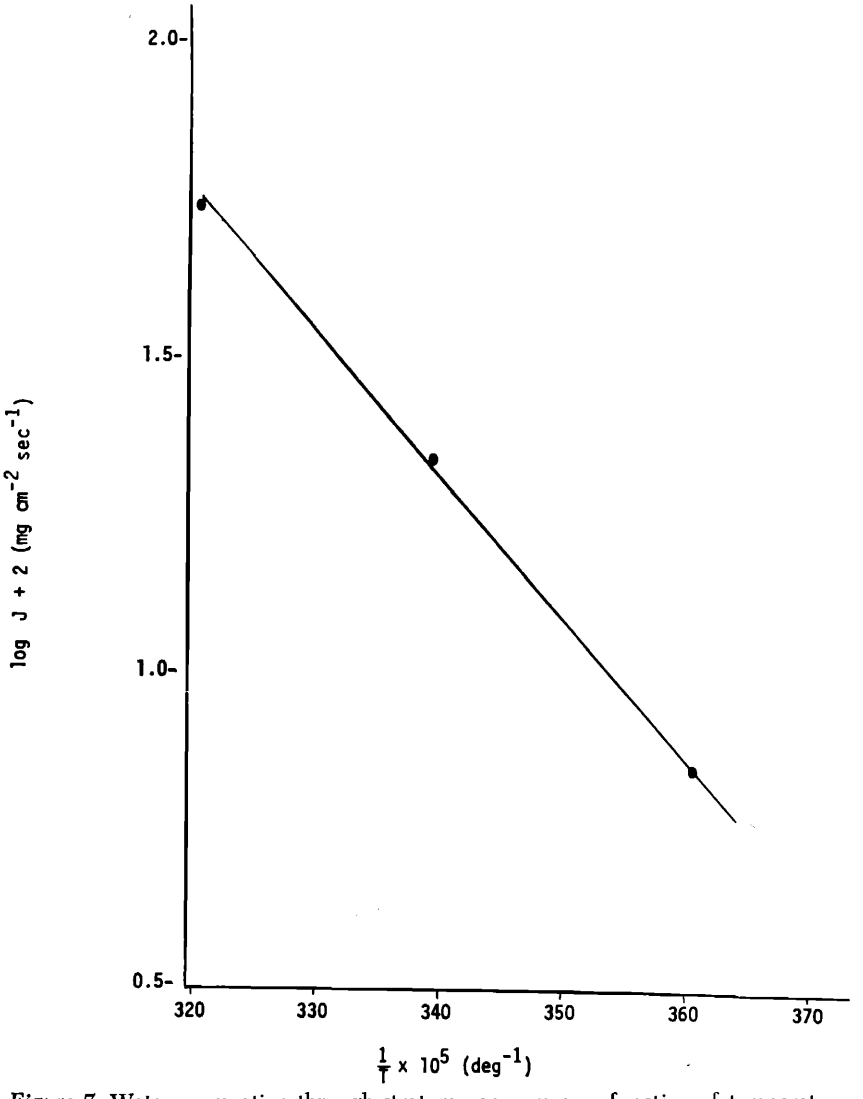


*Figure 7.* Water permeation through stratum corneum as a function of temperature

2. The rate of water vapor absorption of human stratum corneum depends on the relative humidity. The equilibrium water content of extracted and unextracted human stratum corneum at humidities below about 80% is essentially the same.

3. The rate of water vapor transmission through human stratum corneum *in vitro* is a linear function of the ambient relative humidity. This rate is markedly decreased by a reduction in temperature.

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