0022-202X/78/7102-0140$02.00/0
THE JOURNAL OF INVESTIGATIVE DERMATOLOGY, 71:140–144, 1978

Vol. 71, No. 2
Printed in U.S.A.

The Influence of Water Content, Chemical Treatment and Temperature on the Rheological Properties of Stratum Corneum

BARRY F. VAN DUZEE, PH.D.

The Procter & Gamble Company, Miami Valley Laboratories, Cincinnati, Ohio, U.S.A.

The elastic modulus is determined as a function of water content for untreated stratum corneum, and stratum corneum treated with urea and LiBr. The modulus is also determined for stratum corneum at temperatures from 2°C to 45°C and on samples with the water solubles extracted. The modulus is independent of temperature, but may be modified by binding of small molecules to the protein in stratum corneum. The modulus is found to be a function of water content, not water activity.

The study of the rheology of stratum corneum is a study of the ability of that structure to undergo flow or deformation. Most commonly, the ability of stratum corneum to stretch in response to a force is quantitated. This quantity is called the elastic modulus of the stratum corneum. The rheological properties of stratum corneum and their relationship to water content have been of interest mainly because of their supposed relationship to skin condition. Skin that is dry, clinically, is tight and drawn and is thought to lack water. In vitro stratum corneum that has a low ratio of water to stratum corneum is brittle and has a high modulus, thus suggesting that tightness in vivo is indeed merely a manifestation of a physical property of stratum corneum.

Strateum corneum has been shown to absorb large quantities of water. The water in the structure is either associated with the protein and held in a highly immobile state (bound water) or it is associated less specifically with the structural components of the stratum corneum (free water). The water content is known to have a large effect on the modulus of stratum corneum, producing a 2 order of magnitude decrease in the value over a rather small range in water content [1]. Also, extraction of a water-soluble fraction (generally termed Natural Moisturizing Factor, or NMF) from stratum corneum, treatment with certain chemical agents, and variation of the temperature have all been shown to produce changes in the modulus [2–4].

Recently, however, results have been reported which suggest that temperature may not effect changes in the modulus [3]. Also it may be possible that the changes produced by chemical agents are not alterations of the stratum corneum itself, but only alterations of the water binding ability of the stratum corneum. If the modulus is a function of water content, and not relative humidity (thermodynamic water activity), the chemicals may not be producing a fundamental change in the properties of the stratum corneum.

The purpose of this work then is to determine (1) if temperature affects the modulus of stratum corneum, (2) if the modulus is a function of water content or water activity, and (3) if the modulus of stratum corneum may be altered by chemical treatment.

Manuscript received Nov 18, 1977; accepted for publication January 31, 1978.

Reprint requests to: Dr. B. F. Van Duzee, The Procter and Gamble Company, Miami Valley Laboratories, P.O. Box 39175, Cincinnati, OH 45247.

Abbreviation:
NMF: Natural Moisturizing Factor

METHODS

Sample Preparation

Stratum corneum used in these experiments was obtained at autopsy in the form of midline sections. Only female skin was used due to the lower number of hair follicles. The epidermis was separated by heating on a hot plate and the stratum corneum removed by digesting away the epidermis in a trypsin bath. The methods of preparation are described in detail elsewhere [5].

Samples without NMF were prepared by extraction with ether for 5 min at ambient temperature followed by a 24 hr extraction with water, also at ambient temperature.

Thermal Analysis

The thermal analysis procedures used in this work are described in detail elsewhere [5]. Briefly, samples used for thermal analysis were approximately 6 cm^2 in area. They were treated by soaking overnight in water (for controls), or in 3 M solutions of LiBr or urea, then dried, rehydrated, and sealed in a hermetically sealing sample pan. The samples were scanned thermally at 20°C/min from −50°C to 140°C in a Perkin-Elmer DSC-2 Differential Scanning Calorimeter. The denaturation temperatures of proteins and melting temperature of the lipids in stratum corneum were taken as the midpoints of the transitions seen on the thermograms.

Modulus Determinations

Determination of the modulus of elasticity was performed on uniform samples 0.2 cm × 2.0 cm obtained by use of a punch. The samples were stored over anhydrous $CaSO_4$ in individual desiccators until dry and a dry weight was obtained. All weights were taken inside a controlled environment box in which the humidity and temperature were preset.

Samples used as controls were soaked overnight in water and samples used to determine the effects of chemicals were soaked in a 3 M solution of the chemical. The samples were rinsed for 2 min in distilled water after treatment and then replaced in desiccators. Treated dry weights were then obtained and samples were placed in individual hydration chambers (modified weighing jars) over saturated salt solutions chosen to give a specific relative humidity. After equilibration, hydrated weights were obtained and samples were replaced in their hydration chambers and remained there until they were stretched.

Stretching was performed on an Instron Tensile Tester. Samples were removed from their hydration chambers and placed in the fiber clamps of the Instron and stretched at 0.125 cm/min until break. The modulus was determined at the initial hookean region of the stretch. The raw data (slope from the recorder readout) was obtained in units of grams/chart cm. This was related to the modulus as follows:

$$M = \frac{\tau}{\gamma} = \frac{Fl}{A\Delta l}$$

where M is the modulus in dns/cm^2, τ is the stress in dns/cm^2, γ is the strain, Δl and l are the change in length and initial length, respectively, and A is the cross-sectional area of the sample. If the cross-sectional area is assumed constant for a given sample preparation, the initial length the same for each sample, and the force determined as grams, the expression becomes

$$K\frac{MA}{lg} = \frac{m}{\Delta l}$$

where g is the acceleration due to gravity, and m is force in grams, K is a conversion factor, and $m/\Delta l$ is the slope obtained from the raw data. Thus, the slope from the raw data is directly proportional to the modulus. For convenience, the data is reported as

$$K\frac{MA}{lg}.$$

Since no two stratum corneum sample preparations have the same cross-sectional area, the results from one sample preparation of stratum corneum may not be compared directly with the results of another.

The temperature variation experiments were conducted in the same manner as described above except the samples were not soaked in either water or treatment solution and they were maintained at a constant temperature from the time the dry weights were taken until they were stretched.

RESULTS

Modulus Determinations

The results of a modulus determination on an untreated sample of stratum corneum may be seen in Fig 1. Figure 1*a* shows the results as a function of relative humidity and Fig 1*b* as a function of water content (weight fraction of water). The results shown in Fig 1*a* are similar to ones reported by Reiger & Deem [6] in their functional form and the variability in the data is also comparable. When the results are plotted as a function of water content, however, the functional form is, as expected, different (Fig 1*b*).

The results for a sample treated with 3 M urea may be seen in Fig 2. As may be seen by comparison with the control, the urea has the effect of reducing the modulus at all water contents. By using the data gathered during the modulus experiments, the water uptake as a function of relative humidity (water activity) may be determined. As may be seen in Fig 3, treatment with 3 M urea produces stratum corneum which absorbs more water than control at essentially all relative humidities.

Treatment of stratum corneum with 3 M LiBr results in no change of modulus as a function of water content as may be seen in Fig 4. The data is essentially superimposable. However, treatment with LiBr produces stratum corneum which absorbs more water than control at a given relative humidity (water activity) (Fig 5), so the modulus as a function of water activity is actually decreased.

Extraction of the NMF produces stratum corneum which absorbs less water than control. However, the modulus, when compared on a water content basis, is not altered significantly (Fig 6). Of course, a comparison on a relative humidity basis shows a large increase in modulus upon extraction of NMF.

Modulus determinations were performed at 2°C, 25°C, 35°C and 45°C over the entire range of water contents. The results of these experiments may be seen in Fig 7. As the data show, within the uncertainty of the experiment, there is little, if any, difference in modulus at any specific water content over the temperature range of 2°C to 45°C.

Thermal Analysis

Thermal analysis was performed on stratum corneum samples after they were subjected to the treatment soak. Treatment with 3 M urea produces a reduction of 15°C in the denaturation temperature of the nonfibrous intracellular protein component [5] suggesting that the urea is binding to this component of the stratum corneum. The denaturation temperature of the α-keratin is not affected by the treatment nor are the melting temperatures of the 2 lipid components.

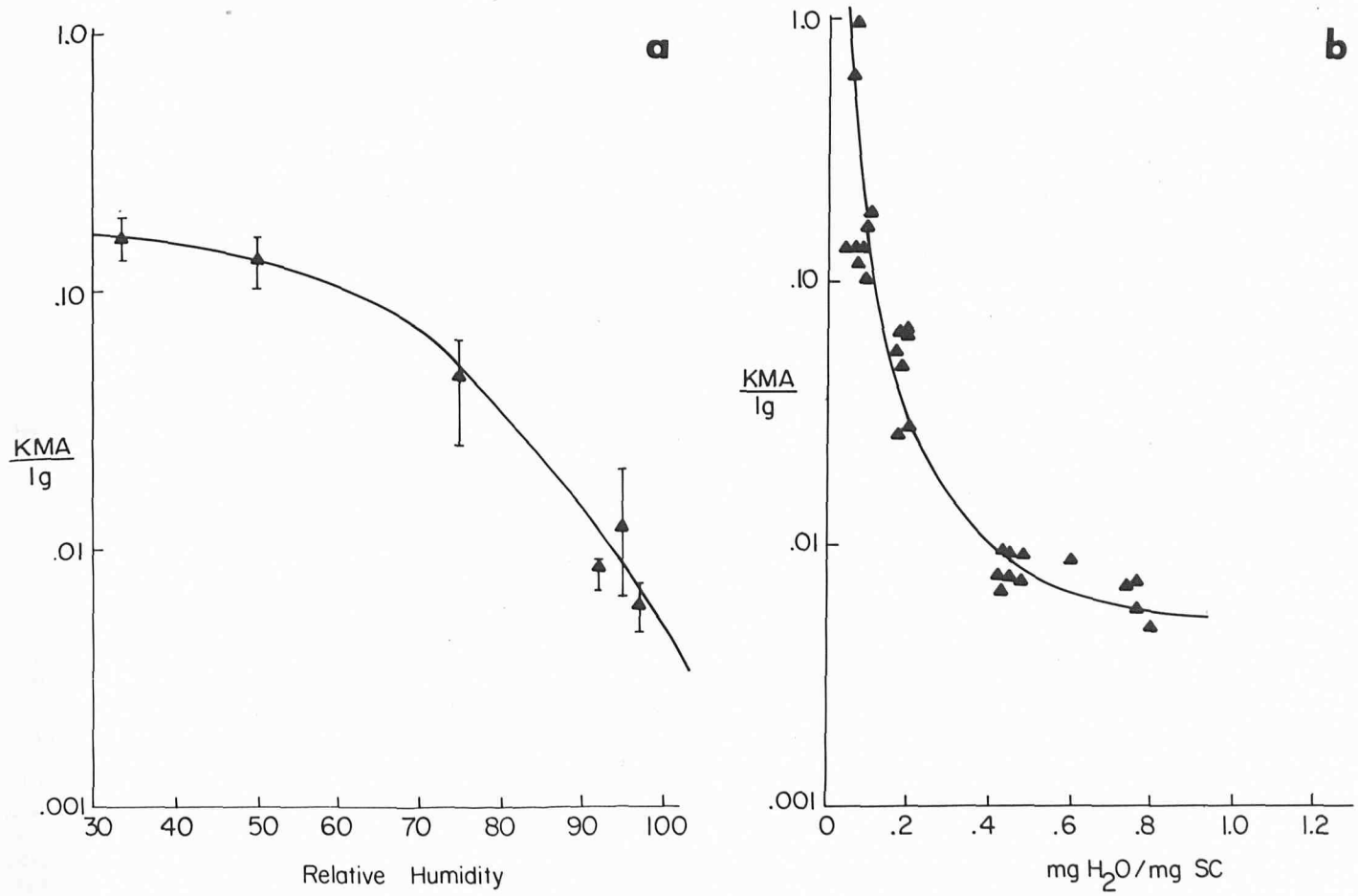

FIG 1. Typical modulus data for a control sample. (*a*) Presented as a function of relative humidity. *Error bars* indicate highest and lowest value obtained at a given relative humidity and the points are averages. (*b*) Presented as a function of water content. The *points* are results from individual samples.

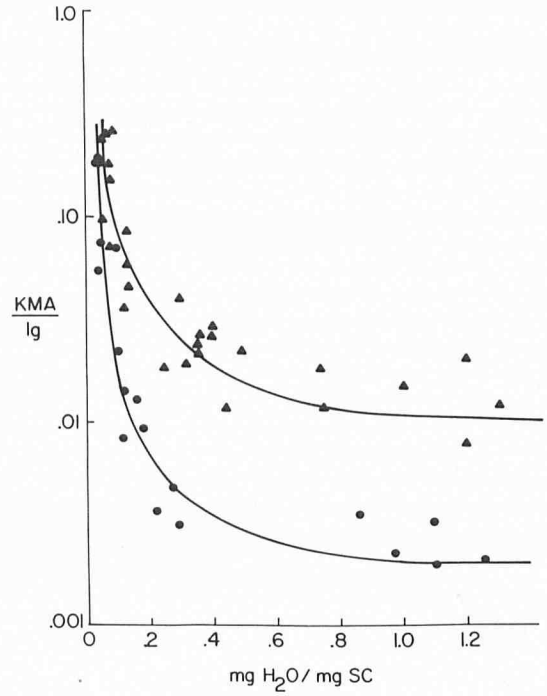

FIG 2. Modulus determination for a control sample (▲) and 1 treated with urea (●).

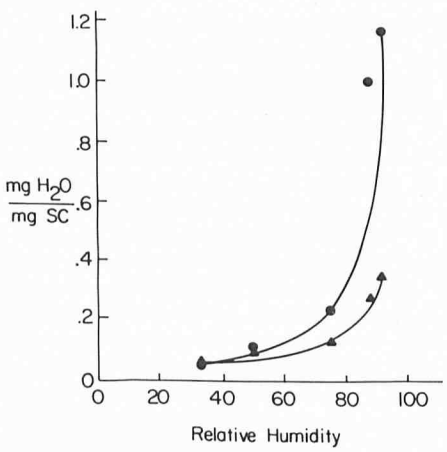

FIG 3. Hydration data showing the effect of urea treatment on the uptake of water by stratum corneum. ▲ = control; ● = urea-treated samples. *Data points* are averages for 4 or more samples.

Treatment with 3 M LiBr has no effect on any of the transition temperatures observed in the stratum corneum which means that, unlike urea, LiBr does not bind to protein in stratum corneum. Extraction of the NMF from stratum corneum by an ether-water extraction procedure also has no effect on the transition temperatures.

DISCUSSION

In the broadest sense, a plasticizer is any compound which enters a polymer matrix and reduces the intermolecular interactions, resulting in a lowering of the modulus and a general softening of the polymer. Commonly, plasticizers are chosen on the basis of their low volatility and effectiveness. That is, the compounds producing the greatest softening for the lowest weight fraction of plasticizer added are preferred. Low volatility insures long life and continued flexibility. The important point is that the effectiveness of a plasticizer is not necessarily a function of its thermodynamic activity, but is dependent on the total amount of plasticizer present in a given volume of polymer. This is expected since the elastic forces in a polymer arise from interchain interactions and the presence of a low molecular weight material in the matrix disrupts these forces. The larger the number of plasticizer molecules present, the more the attractive forces are disrupted, and hence, the dependency on total amount of plasticizer present.

This dependence of the modulus on the content of plasticizer is observed in biological systems as well. For instance, water plasticizes rat tail tendon. The weight ratio of water-to-tendon (swelling ratio) may be altered by changing the pH of the swelling medium and if the modulus is determined at the same time, it is found that by increasing the swelling the modulus is reduced [7].

If water is acting as a plasticizer on stratum corneum, then the modulus should be a function of the amount of water present and not necessarily its activity. Evidence which suggests that the modulus is a function of water content and not activity comes from the LiBr experiments.

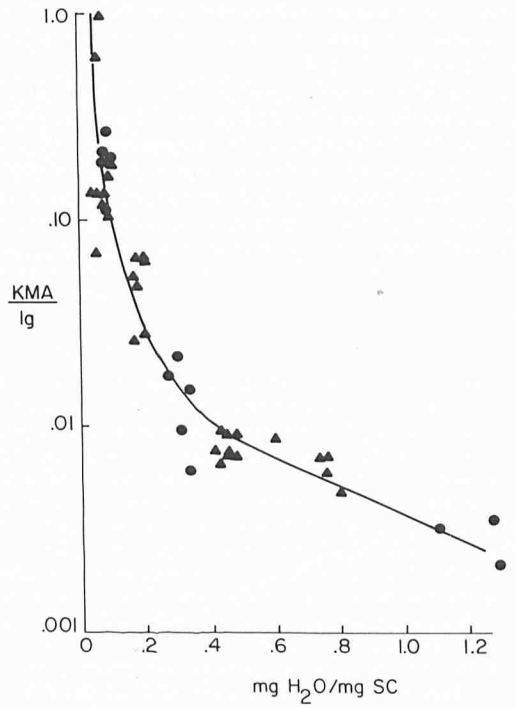

FIG 4. Modulus determination for a control sample (▲) and 1 treated with 3 M LiBr (●).

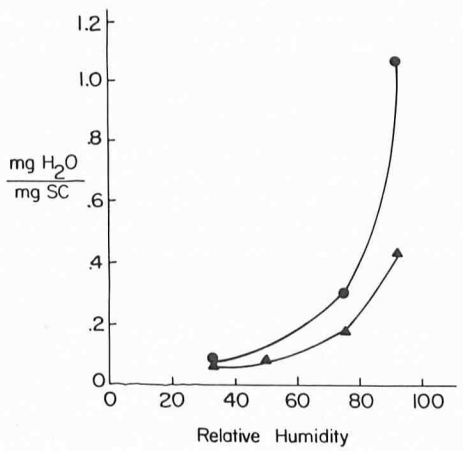

FIG 5. Hydration data showing the effect of LiBr treatment on the uptake of water by stratum corneum. ▲ = control; ● = LiBr-treated samples. *Points* are averages of 4 or more samples.

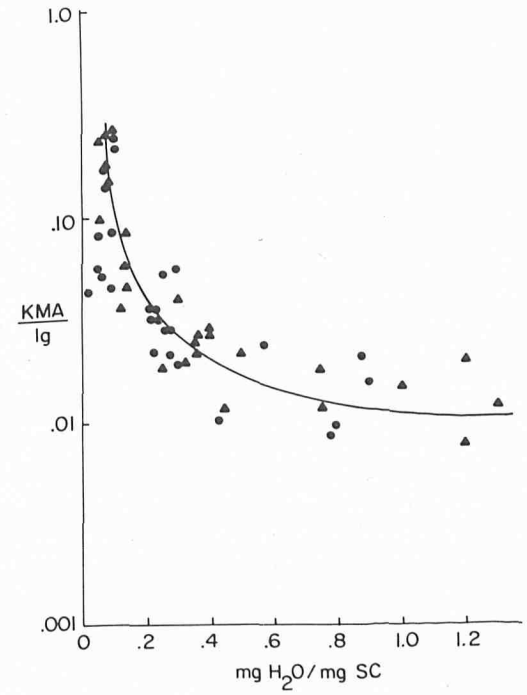

FIG 6. Modulus determination for a control sample (▲) and a sample with water solubles extracted (●).

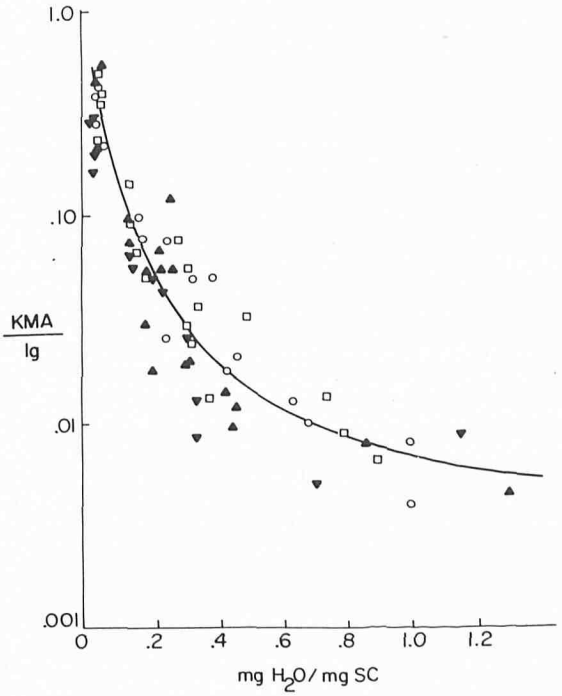

FIG 7. Modulus determination for samples at different temperatures. ○ = 2°C, □ = 25°C, ▲ = 35°C, and ▼ = 45° C.

LiBr treatment increases the amount of water the stratum corneum absorbs over control at any given water activity (relative humidity). The absence of shifts in T_m (melting temperature) determined by thermal analysis for the lipids in stratum corneum indicates the LiBr is not partitioning into the lipid phase and the lack of T_m shift for the protein components in stratum corneum indicates LiBr is not binding to the proteins. Since LiBr does not interact strongly with the stratum corneum, but does increase the hydration, it must be adsorbing on the surfaces in the system and increasing the hydration by nature of its own hygroscopicity.

Further, an examination of Fig 4 shows that at any given water content, the modulus is the same for the control and LiBr-treated samples. Because of the presence of LiBr in the stratum corneum, the activity of the water in the LiBr-treated sample, at any given water content, must be lower than the activity of the water in the control at the same water content. However, the modulus is the same in both systems at any given water content. Thus, it appears that the modulus is a function of water content and not water activity. For this reason then, reports which suggest that the modulus of stratum corneum may be modified should be re-evaluated. If the modulus in these reports is determined at a constant relative humidity rather than a set water content, the changes observed may be due to the presence of hygroscopic materials effecting the hydration of the stratum corneum and not to any fundamental changes produced in the stratum corneum.

The modulus of other protein systems may be modified, apart from changes produced in swelling, by binding small molecules to the protein structure. For instance, the presence of potassium thiocyanate in a swelling medium for rat tail tendon decreases the modulus [8]. This suggests that the modulus of stratum corneum might be modified if small molecules could be bound to the protein in the stratum corneum. The thermal analysis results from urea-treated samples show that the denaturation temperature of a protein component in the stratum corneum is reduced by the urea treatment. This reduction indicates that the urea is binding to the protein in stratum corneum [9], and it might be expected that the modulus of stratum corneum is reduced by this binding. This is indeed the case as is seen from the data in Fig 2. At any given water content, the modulus is lower for the urea-treated samples than for the controls. Since the comparison is made on the basis of a constant amount of plasticizer (water), it may be concluded that urea actually alters the modulus of the stratum corneum, and that this modification is a result of binding of urea to the protein in the stratum corneum.

The NMF extraction experiments are interesting in the light of the urea results. NMF extraction has no effect on the thermal properties of the stratum corneum. This indicates that there is very little, if any, binding of the NMF to the protein occurring in the stratum corneum as opposed to what has been suggested previously [10]. Also, when compared on the basis of water content, extraction of the NMF has virtually no effect on the modulus of the system (Fig 6). These results are essentially identical to the LiBr results and suggest that the presence of NMF reduces the modulus at a given relative humidity, in much the same manner that LiBr does. That is, the NMF, due to its natural hygroscopicity, increases the amount of water the stratum corneum absorbs at any given water activity, but does not significantly interact with the protein in the stratum corneum.

Up to this point, stratum corneum appears to behave in every way similar to fibrous protein systems in terms of its rheology. Thus, it might be expected that the effects of temperature on stratum corneum are the same as for other fibrous proteins, as indeed it is.

The modulus for elastin is essentially invariant from 0.5°C to 50°C when fully swollen [11]. The results of the temperature variation experiments on stratum corneum show that from 2°C to 45°C, over a full range of water contents, the modulus is nearly invariant (Fig 7).

Reiger and Deem [3] found essentially the same results. Their lowest temperature experiment showed some increase in modulus, which may be due to changes in the rate of diffusion of water into the stratum corneum at such low temperatures. Wilkes, Brown, and Wildnaver [12], however, found that at temperatures above 30°C that the modulus dropped by two orders of magnitude. Before determining the modulus, the samples were soaked in water at the appropriate temperature for 2 minutes and then stretched. They suggested that the drop might be due to temperature-induced phase changes in the lipid

structure or to increased diffusion of water into the stratum corneum. Based on the fact that the stratum corneum is in every way behaving as other fibrous protein systems, and the experimental results presented herein, it appears that their observed modulus changes were due to increased diffusion of water into the stratum corneum.

In conclusion, stratum corneum may be thought of as behaving as a polymeric system in terms of its rheological properties. The modulus; (1) is dependent on the content, not the activity, of water in the system, (2) may be modified by binding small molecules to the protein structure, and (3) is independent of temperature from 2°C to 45°C.

REFERENCES

1. Park AC, Baddiel CB: Rheology of stratum corneum. I. A molecular interpretation of the stress-strain curve. J Soc Cos Chem 23:3–12, 1972
2. Park AC, Baddiel CB: Rheology of stratum corneum. II. A physiochemical investigation of factors influencing the water content of stratum corneum. J Soc Cos Chem 23:13–21, 1972
3. Reiger MM, Deem DE: Skin moisturizers. II. The effects of cosmetic ingredients on human stratum corneum. J Soc Cos Chem 25:253–262, 1974
4. Middleton JD: The effects of temperature on isolated stratum corneum and its relationship to skin chapping. Br J Dermatol 81:717–721, 1967
5. Van Duzee BF: Thermal analysis of human stratum corneum. J Invest Dermatol 65:404–408, 1975
6. Reiger MM, Deem DE: Skin moisturizers. I. Methods for measuring water regain, mechanical properties, and transepidermal moisture loss of stratum corneum. J Soc Cos Chem 25:239–252, 1974
7. Ciferri A, Rajagh LV, Puett D: Interaction between proteins and salt solutions. III. Effect of salt type and concentration on the shrinkage temperature. Biopolymers 3:461–480, 1965
8. Puett D, Ciferri A, Rajagh LV: Interaction between proteins and salt solutions. II. Elasticity of collagen tendons. Biopolymers 3:439–459, 1965
9. Mandelkern L, Stewart WE: The effect of neutral salts on the melting temperature and regeneration kinetics of the ordered collagen structure. Biochemistry 3:1135–1137, 1964
10. Park AC, Baddiel CB: The effect of saturated salt solutions on the elastic properties of stratum corneum. J Soc Cos Chem 23:471–479, 1972
11. Hoeve DAJ, Flory PJ: The elastic properties of elastin. J Am Chem Soc 80:6523–6526, 1958
12. Wilkes GL, Brown IA, Wildnauer RH: The biomechamical properties of skin. CRC Critical Rev Bioeng 1:453–495, 1973

Announcement

The Bureau of Laboratories of the Center for Disease Control, Atlanta, Georgia will offer a series of laboratory training courses of varying duration (2 days to 2 weeks) in specific areas beginning on July 10, 1978 through June 22, 1979. Information and application forms may be obtained from the Registrar, Bureau of Laboratories, Center for Disease Control, Atlanta, Georgia 30333.