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Mechanical properties of dry, normal, and glycerol-treated skin as measured by the gas-bearing electrodynamometer

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Synopsis

The viscoelastic properties of dry, normal, and glycerol-treated skin of the lower leg have been measured with a gas-bearing electrodynamometer (GBE). Elastic modulus measurements are shown to correlate well with visual assessment of the skin condition by a trained dermatological grader. Dry skin is generally stiffer than normal skin and glycerol treatment can indeed soften the skin. Removal of the outer layers of the stratum corneum by tape stripping resulted in an almost 50% reduction in the moduli—indicating a significant contribution to the mechanical properties of the skin from these layers as measured by the GBE.

INTRODUCTION

A variety of methods for characterizing tissue mechanical properties have been applied to skin. However, when the objective is to measure the elastic properties of stratum corneum, care must be exercised in the choice of proper instrumentation and the design of appropriate test methodology. Classical stress-strain measurements can be used to characterize strips of isolated stratum corneum under tension (1,2). Most *in vivo* tensile measurements involve rather large displacements and are certainly influenced heavily by the skin layers to which the stratum corneum is attached (3–5).

This is especially true when the stress is applied perpendicular to the skin. Indeed, one major application of such techniques as indentometry and levarometry (6), ballistometry (7), and suction methods (8,9) is the study of age-dependent changes of the dermis (10,11). More sophisticated experiments measuring the mechanical response of skin as a function of frequency have the advantage that small amplitudes may be used, either perpendicular (12) or parallel (13–15) to the skin surface. These techniques show promise in being easy to use and possibly able to separate dermal and epidermal response components at different frequencies (14), with a little interpretation.

The two techniques which probably provide the most direct information regarding stratum corneum mechanical properties are the low-torque torsional method of Rigal and Leveque (16) and the gas-bearing electrodynamometer (GBE) of Christensen *et al.* (17,18). We have used a dynamometer identical to that of Christensen *et al.* in our studies. The GBE measures the displacement of skin in response to a sinusoidal driving force. The force coil and displacement measurement core are mounted on the same

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armature, which is suspended upon a highly compliant, virtually frictionless bearing of pressurized gas. These properties enable the measurement of viscoelastic response of very soft tissues under conditions of very low driving forces and displacements.

Although the technique does not measure a fundamental elastic property of stratum corneum, it does provide a convenient and rapid way to determine the effect of various agents on its mechanical properties (19,20). For example, a factor of two decrease in the elastic modulus can be observed immediately after the application of water to the skin. Thus, one has evidence that the stratum corneum contributes significantly to the elastic modulus, since a rapid response would not result from the action of water on the dermis.

We have applied the GBE technique to measure the mechanical properties of skin having varying degrees of dryness, and to determine the effect of humectants such as glycerol (1,2,3-propanetriol) on the mechanical properties of skin. The results of these studies are reported in the following sections.

MATERIALS AND METHODS

CLINICAL GRADING AND GLYCEROL TREATMENT

We report data from two separate clinical experiments. In the first experiment, the outer portion of the lower part of the left leg (28 female panelists) was graded by two trained judges according to the scale in Table I prior to measurement with the GBE. Grading was an average over the entire area rather than the small site measured instrumentally. The purpose of this first experiment was to determine how well the GBE elastic modulus correlates with visual skin grade.

In a second investigation, five female panelists applied 0.6 ml of 40% glycerol solution to a 100 cm^2 area on one calf (outer aspect) and the same amount of water to the other calf. Treatments were applied once in the morning and once four hours later in the afternoon for three days, omitting the last treatment. Visual grade assessments and GBE measurements were made immediately prior to each treatment. The treatment sites were graded by a single trained judge prior to measurement with the GBE. Panelists followed their usual bathing/showering habits throughout the study. The panelists were assumed to have achieved equilibrium with the room temperature and humidity, which were 72°F and 54 ± 3%, respectively, throughout the study.

EQUIPMENT AND MEASUREMENT PROCEDURE

The electrodynamometer is basically that described by Christensen *et al.* (17) and Hargens (18). For *in vivo* measurements, the GBE head is supported in a three-dimen-

Table I
Leg Grading Scale*

0	Skin smooth and lustrous, no detectable dryness or follicle irritation
1	Skin lustrous, slight ashing visible in cracks
2	Skin dry, smooth, but without lustre, moderate ashing covering general surface area
3	Skin slightly rough overall with slight scaling; high amount of ashing covering total leg area
4	Skin has moderate to high roughness, moderate scales formed with some small, fine cracks; high ashing overall
5	Skin is rough with much scaling; large cracks with high ashing overall

* ½ grade units are allowable.

sional translation stage firmly mounted to a Ralmike's portable toolmaker's bench (South Plainfield, N.J., Catalogue #085-3). This arrangement allows for maximum mechanical stability, required for accurate stress-strain measurements, and simultaneously provides a portable yet secure work surface for mounting the electronics (Figure 1). A Tektronix FG-501A function generator, slightly modified to enable accurate adjustment of the D.C. offset using an auxillary 10-turn potentiometer, was used to drive the force coil of the GBE head at a sinusoidal frequency of 2 Hz. The other electronic components are analogous with the description by Christensen and Hargens (17,18). One major improvement is the use of a digitizing oscilloscope (Tektronix 5223 with 5B25N time base generator) for capturing force and displacement traces. These traces are transferred to a Tektronix 4051 computer equipped with a signal processor (4051R07) to enhance data analysis. Every eighth point of each trace was recorded on magnetic cartridge tape for later analysis.

For instrumental measurements, panelists would lie on their side on a portable cot, and were requested to remain as still as possible. Attempts to restrain movement of the leg with straps were abandoned when it became apparent that this method applied

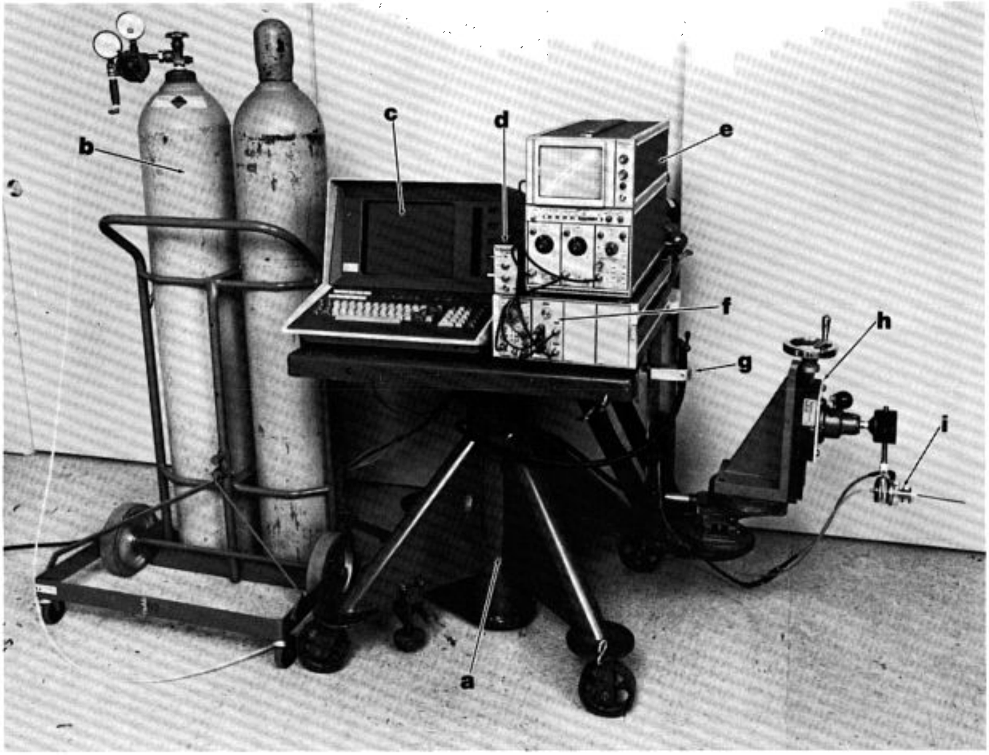

Figure 1. *In vivo* GBE Apparatus. a) Ralmike's portable toolmaker's bench #085-3. b) Nitrogen tank (regulated to 5 psi). c) Tektronix 4051 computer. d) Shaevitz CAS-025 signal conditioner for LVDT. e) Tektronix 5223 digitizing oscilloscope. f) Tektronix FG-501A function generator with modified offset potentiometer. g) Control for translation parallel to GBE axis. Cable drives one axis of Ralmike's 023-700R positioning table. h) Ralmike's #060-2-2 right-angle lead screw slide. i) GBE head mounted in a swivel lock attached to the lead screw.

unknown tension to skin in the measurement area and actually increased panelist movement due to discomfort.

Contact between the dynamometer and the skin was achieved with double-sided tape (3M, No. 666®). A circular disk of tape of diameter 4 mm was placed on the skin and a bushing insulator (Amphenol 31-1099), a plastic disk with a hole drilled through the center, was placed on top of the tape. A wire probe (from the armature) was bent 90° to form a hook which fits into the bushing insulator. The possible influence of tape occlusion at the test site was ignored since the viscoelastic response appeared to be independent of time.

The elasticity values for the first experiment represent averages of three traces from one site of the lower left leg. In the glycerol treatment study, the GBE measurements represent averages of two traces obtained from three sites on each leg, approximately 10, 15, and 20 cm below the knee joint, centered on an axis through the knee and ankle joints. Four fiducial marks on the calf outlining the rectangular treatment area were used with a template to place the GBE probe to within 1 cm of each of the three measurement spots (see Figure 2). No template was used to position the probe in the first study.

The force coil driving amplitude was adjusted at each measurement site to provide a maximum armature displacement of only ±0.75 mm from equilibrium in an effort to minimize the contribution of nonlinear viscoelastic response, as will be discussed later. The oscilloscope was operated in left-versus-right mode, displaying stress-strain ellipses which varied slightly with time due to panelist movement and respiration. The operator would examine a series of traces with time and save only the traces which formed closed loops with symmetric shapes.

Data Analysis. An oscillating stress (force) is applied to the skin and the resulting strain (displacement) follows with a certain lag or phase shift (δ). For a linear response, one writes the stress as:

$$\sigma = \sigma_0 \sin(\omega t + \delta) \tag{1}$$

and the strain as

$$\epsilon = \epsilon_0 \sin(\omega t) \tag{2}$$

If the stress is written as

$$\sigma = \sigma_0 \cos\delta \sin\omega t + \sigma_0 \sin\delta \cos\omega t, \tag{3}$$

the elastic or shear storage modulus is defined as (21)

$$E' = \frac{\sigma_0}{\epsilon_0} \cos\delta \tag{4}$$

and the viscous or shear loss modulus is defined as

$$E'' = \frac{\sigma_0}{\epsilon_0} \sin\delta \tag{5}$$

Thus, from a plot of ϵ and σ vs. time, one can determine σ_0, ϵ_0, and δ. This type of analysis can be done with use of the time base generator to obtain stress and strain as a function of time.

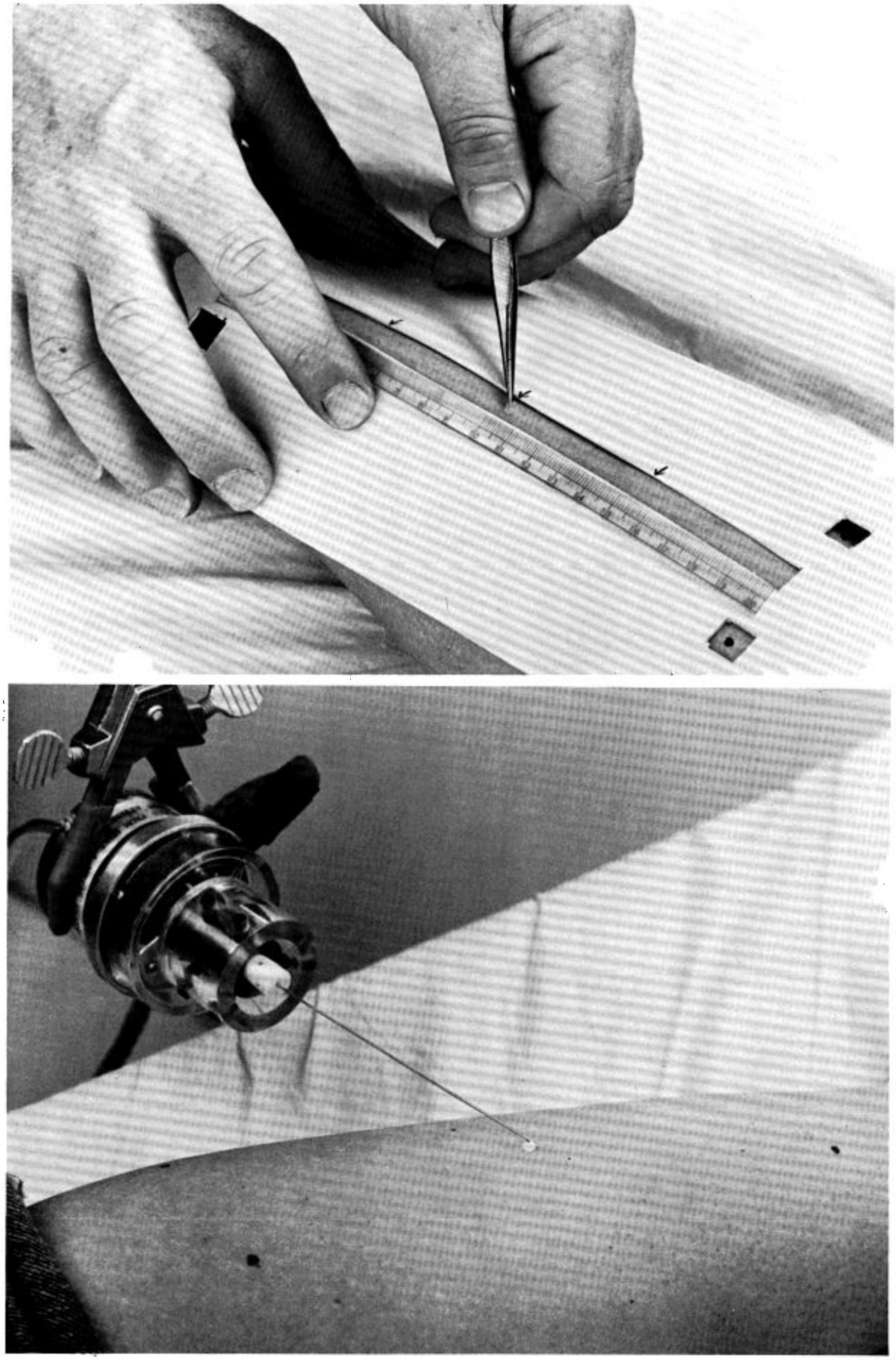

Figure 2. Placement of GBE probe for measurement on lower leg sites. a) Positioning of 4-mm double-sided adhesive tape circle by means of a template. b) GBE attached at the central site. Three of the four fiducial marks which outline the treatment site and which are used to position the template are visible.

An alternate way to obtain the moduli is from a plot of σ vs. ϵ, where one has

$$\sigma = E' \epsilon + E'' (\epsilon_0^2 - \epsilon^2)^{1/2} \tag{6}$$

and the shape of the curve is that shown in Figure 3 for $\epsilon_0 = \sigma_0 = 1$ and $\delta = 60°$. The elastic modulus is given by

$$E' = \frac{\sigma(\epsilon = \epsilon_0)}{\epsilon_0} \tag{7}$$

where it should be noted that the slope of the major axis of the ellipse is not identical with E′. The loss modulus is given by

$$E'' = E'\epsilon'/(\epsilon_0^2 - \epsilon'^2)^{1/2} \tag{8}$$

or by

$$E'' = \frac{\text{area of the loop}}{\pi \epsilon_0^2} \tag{9}$$

where ϵ' is the displacement at zero force.

Equations (7) and (8) are easily used with the computer to find maxima, minima, and crossing points.

RESULTS AND DISCUSSION

The elastic modulus as measured by the GBE is quite sensitive to water, as is shown in Figure 4. Water was applied from a squirt bottle with the probe attached. The loop

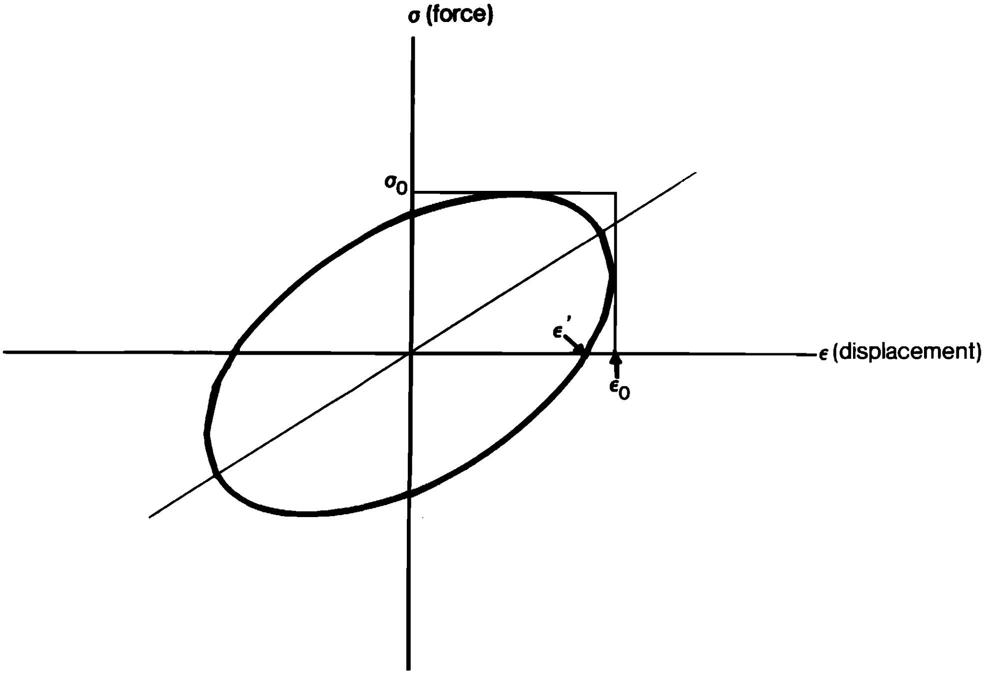

Figure 3. Force vs. displacement ($\sigma_0 = \epsilon_0$, $\delta = 60°$) for linear response.

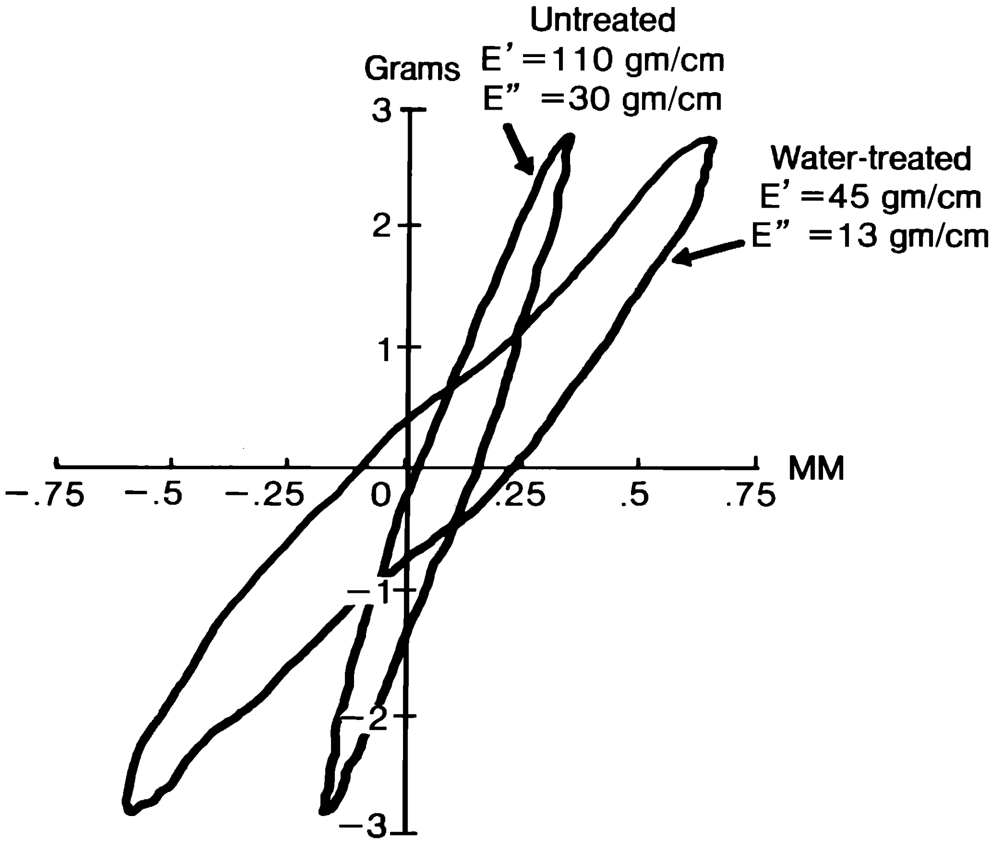

Figure 4. Effect of water on viscoelasticity of human skin (back of hand) *in vivo* (force vs. displacement) at 20% relative humidity.

shape reached equilibrium within minutes. The loss modulus is also lowered by water and the ratio E′ to E″ remains essentially constant. The outer layers of the stratum corneum contribute significantly to the mechanical properties of the skin, since the moduli can be reduced by almost 50% with four tape strips as shown in Figure 5 for a single individual. (Here the amount of skin removed was measured by Lowry (22) protein analysis).

These elastic storage modulus results are similar to those of Christensen *et al.*, who also observed a reduction in E′ upon applying water and lotions to the skin or upon tape stripping (17). However, the loss modulus results presented here are in contradiction with the behavior of their analogous parameter θ. They found θ to increase, rather than decrease, upon water treatment, or upon making progressively smaller circumference circular incisions through the epidermis surrounding the probe site.

We suggest this discrepancy may largely be a result of the differences in maximum probe displacement used in the two studies. Christensen *et al.* cite a typical skin displacement of 3–4 mm, which is up to five times greater than our maximum displacement of ±0.75 mm. We have found that larger displacements form nonelliptical stress-strain curves, which exhibit preconditioning and stress relaxation effects, all hall-

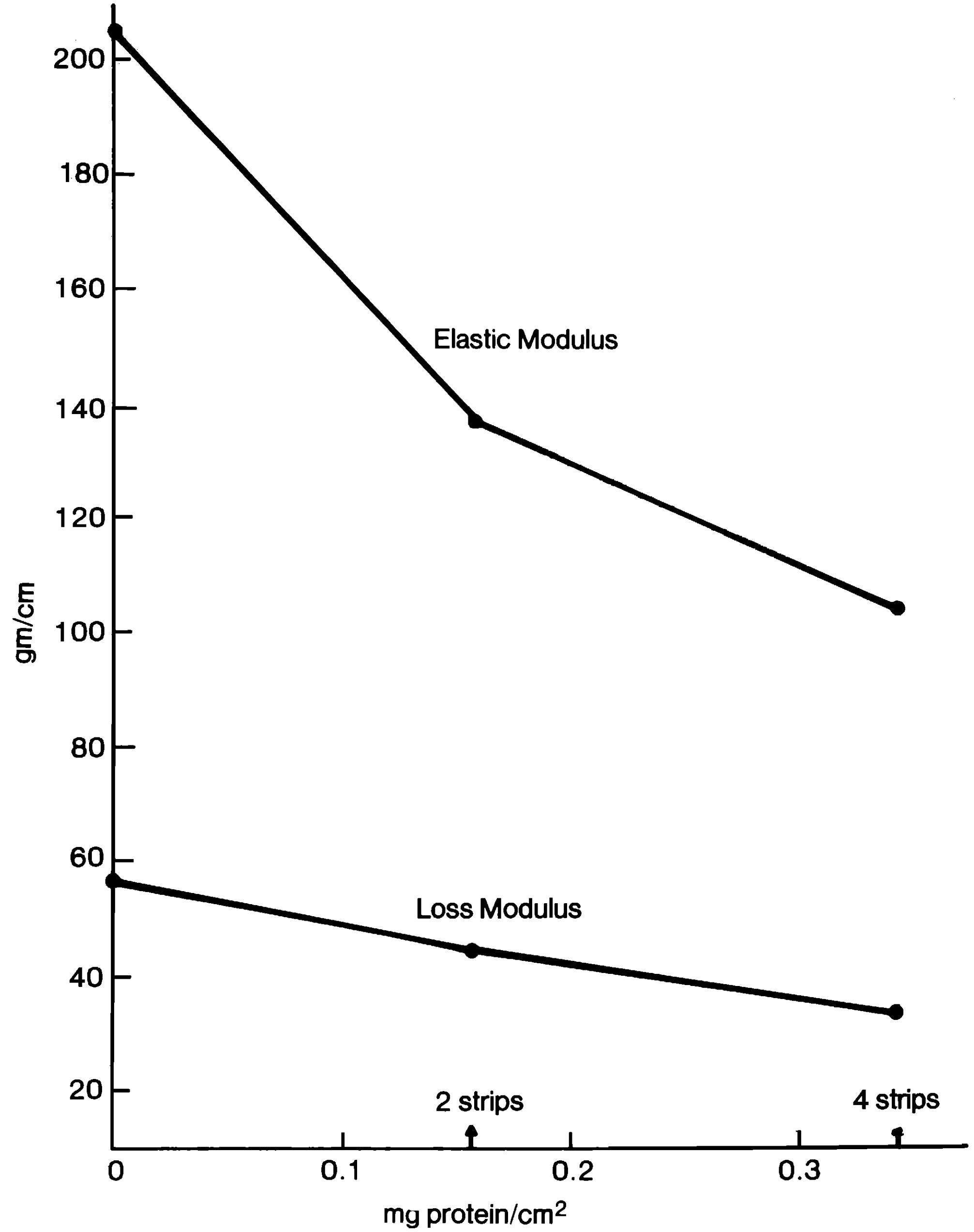

Figure 5. Effect of tape stripping on mechanical properties of skin (lower leg) at 29% relative humidity. The abscissa represents the amount of protein removed from skin by the tape strips.

marks of nonlinear viscoelastic behavior (23–26). For example, the water-treated trace in Figure 4 displays a slightly nonlinear response. Many of the traces shown in the work of Christensen *et al.* display a higher degree of nonlinearity.

For larger displacements, the slope and precise shape of the trace depend upon both

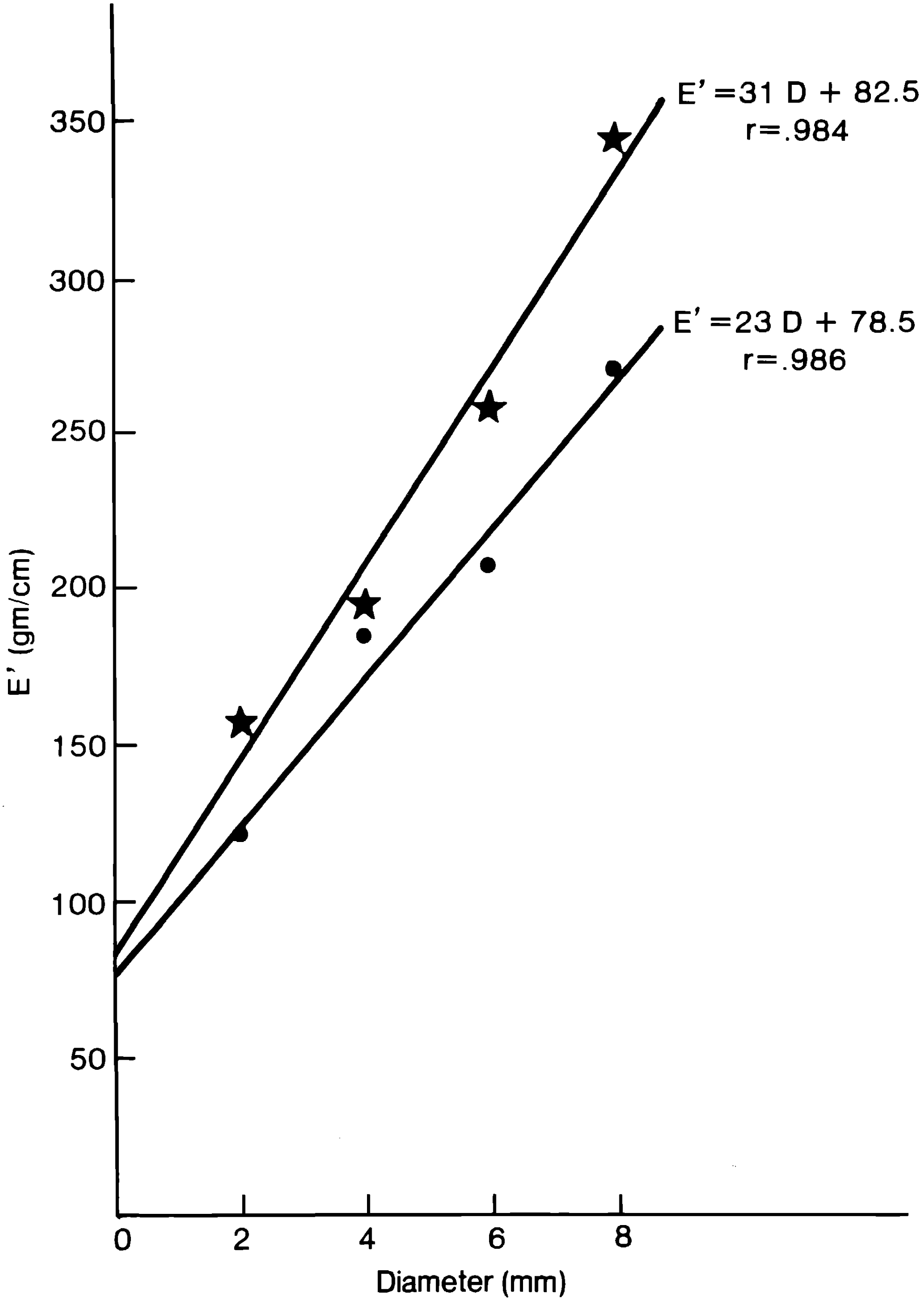

Figure 6. Effect of tape diameter (D) on the elastic modulus of skin (lower leg). Data are for two different subjects.

Table II
Variation in E′ with Vertical Position Along the Lower Leg, Outer Aspect of the Calf (Mean Values for Five Panelists Selected for Dry Skin)

Distance below knee joint	Glycerol-treated Sites		Water-treated Sites	
	Initial	Final	Initial	Final
10 cm	207 ± 71	142 ± 49	149 ± 55	178 ± 72
15 cm	353 ± 119	252 ± 55	304 ± 99	319 ± 93
20 cm	451 ± 46	313 ± 94	415 ± 166	448 ± 184

amplitude and driving frequency in a complex manner, and the analysis procedure derived above is no longer valid. A theory describing the nonlinear behavior of stratum corneum under uniaxial tension *in vitro* has been presented recently (26). This approach would not be tractable for dynamic viscoelastic measurements such as ours. Rather, it is appropriate to apply harmonic analysis to the displacement and to apply equations

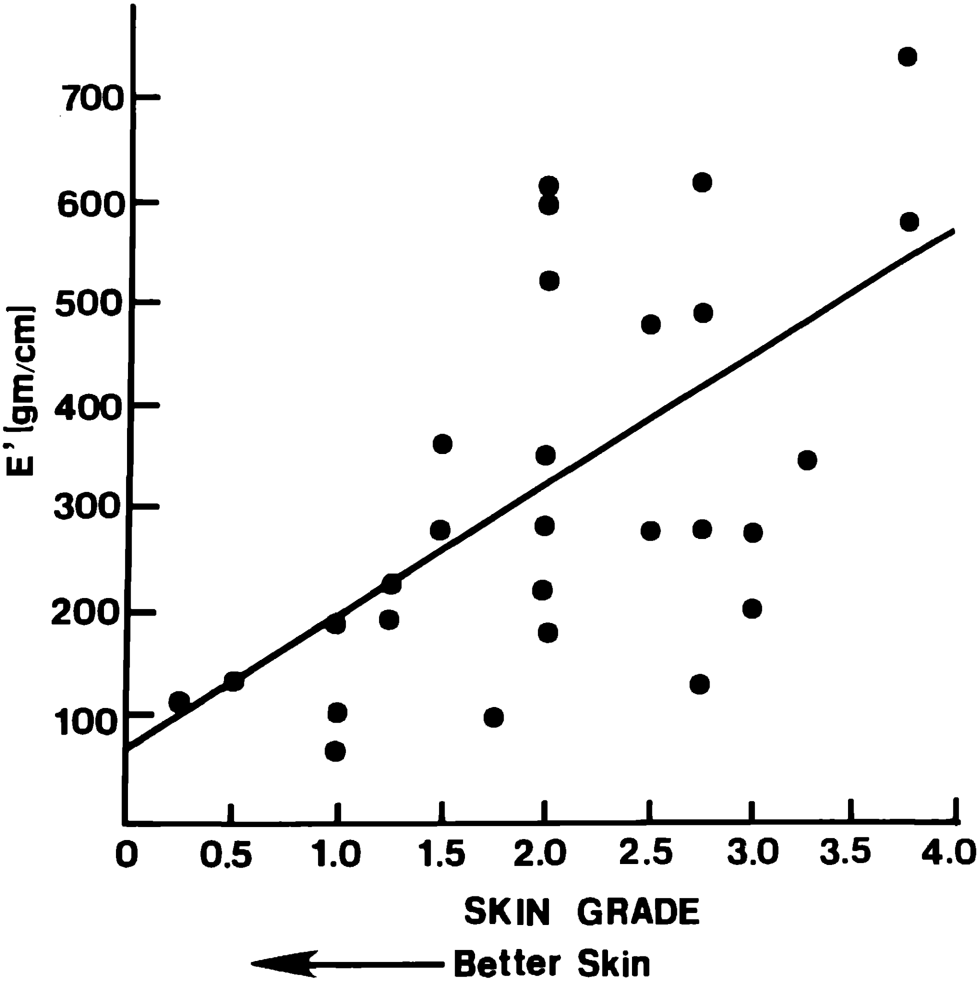

Figure 7. Elastic modulus of skin (lower leg) vs. skin grade.

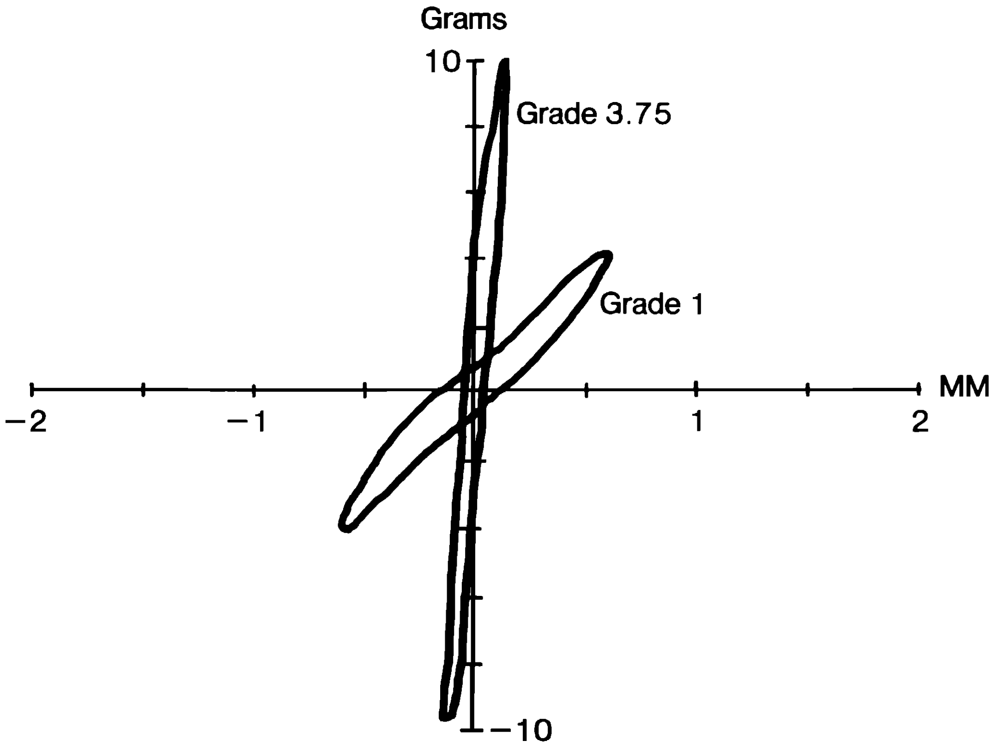

Figure 8. Stress-strain loops for soft and stiff skin (lower leg).

(1–9) above to the fundamental component (23). The presence of higher odd harmonics, when not accounted for in this way, will result in large errors in measurement of the loss modulus and probably much smaller errors in the storage modulus. It was not necessary to use harmonic analysis for the range of displacements encountered in the studies presented here. We believe that nonlinear viscoelastic response may indicate a stronger dermal contribution to the measurement than is present during linear viscoelastic response (4,5).

The elastic modulus is linear with respect to the diameter of the tape disk as is shown in Figure 6 for two different subjects. This seems odd at first, since the elastic modulus is defined in such a way as to be independent of sample dimensions. This functional dependence of E′ on disk diameter is a consequence of the fact that we have no control over the dimensions of the skin that are involved in the movement of the probe. In fact, the E′ we measure is not equivalent with but only analogous to an elastic modulus, since the area cannot be defined *in vivo* for a formal definition of the applied stress. Therefore we report E′ in units of force (gm) per unit displacement (cm). The linear dependence seems intuitively to be due to the fact that only the stratum corneum around the circumference of the disk is being stretched along the projection of motion. The stratum corneum attached to the tape is not being stretched and does not contribute to the measurement. The non-zero intercept reflects to some degree the contribution from the surrounding skin or underlying tissue.

The mechanical properties are not very sensitive to the angle between the probe and the leg, but abrupt changes in the stress-strain loop can be observed and easily identified

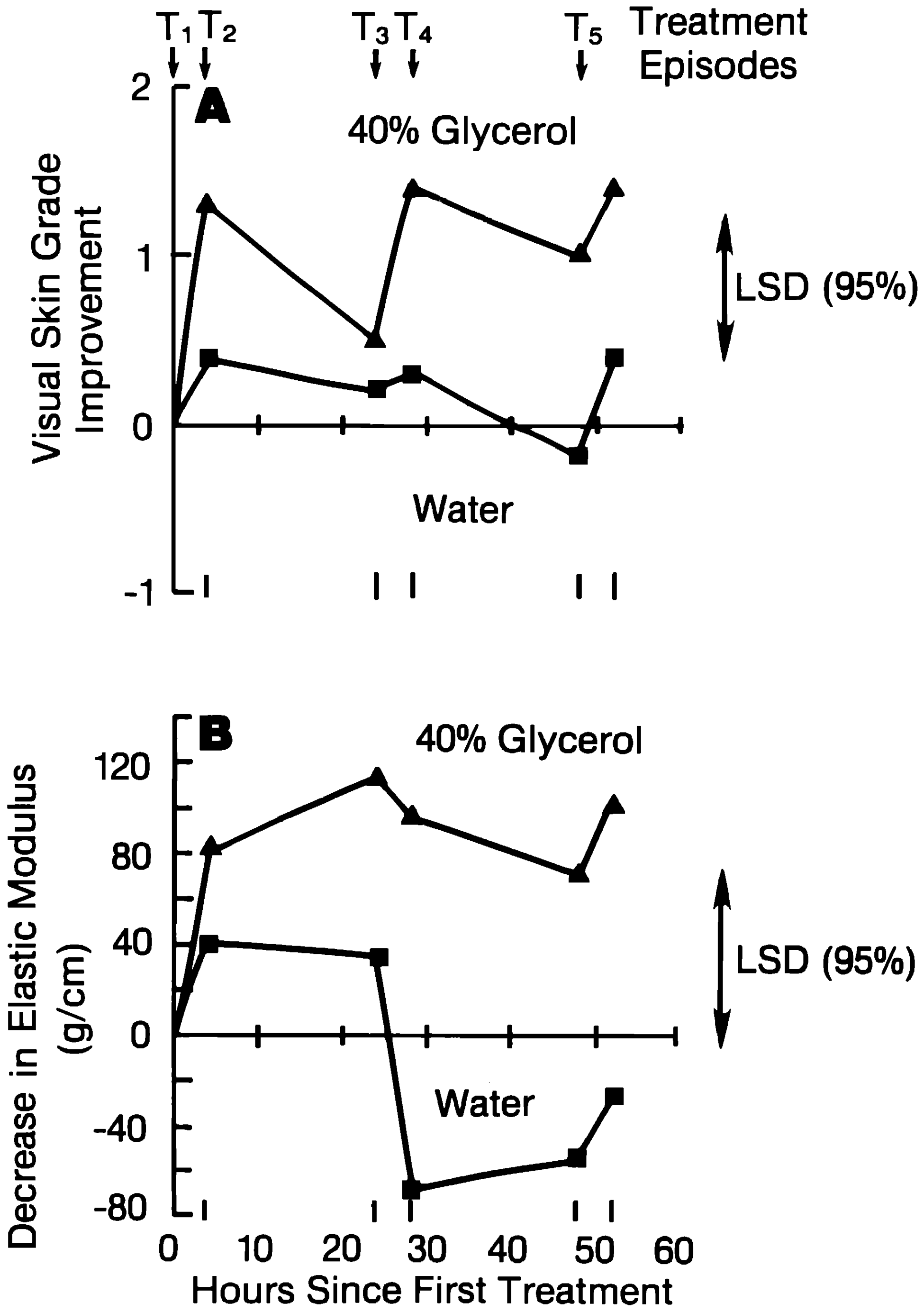

Figure 9. Effect of progressive treatments of 40% glycerol (△) and water (□) on: a) Visual skin grade improvement. Larger numbers imply that skin appears less dry and more normal. b) Decrease in elastic modulus E1. Larger numbers imply that skin is less tight and more flexible. The least significant difference (LSD) for 95% confidence is indicated for each panel.

if the probe alignment becomes too poor. As one progresses down the outer leg (below the knee), the elastic modulus generally increases even as much as a factor of two (Table II). (One also observes that the skin condition becomes poorer further down the leg.) Sizable differences in elasticity can be observed for distances as close as 3 cm.

A comparison between skin grade and elastic modulus is shown in Figure 7, where one observes that increasing skin grade usually corresponds to stiffer skin or increasing elastic modulus. The correlation coefficient is $r = 0.59$ with $p = 0.001$, where p is the probability of observing that large of a sample correlation coefficient if, in fact, the true correlation is zero. Although the probe was attached at approximately the same relative position on each panelist, some of the differences may be due to differences in position. All of the 28 subjects were female and ranged in age from 25–55, except for one subject who was five years old. Her stress-strain loop for grade 1 is compared in Figure 8 with an adult's skin of average grade 3.75. The contrast is rather dramatic.

The effect of glycerol treatment on the visual appearance and elastic properties of lower leg skin is shown in Figure 9. Plotted are the mean decrease in visual skin grade and in E1 relative to the first measurement. The data exhibit that glycerol softens skin (lowering the elastic modulus E1) while providing a visual benefit relative to water treatment. During the first 24 hours, the visual benefit appears not to last as long as the softening benefit. Much of the visual benefit is lost 18 hours after the second treatment (24 hour time point), whereas the GBE detects a significant difference in skin softness at this same time (99% confidence level). Beyond the 28-hour time point, the visual and mechanical measurements follow roughly similar patterns for each treatment. In fact, the absolute visual grade and E1 values for this experiment correlate well with each other ($r = 0.65$ with $p = 0.02$), lending support to the notion that similar benefits are being measured by the two techniques.

CONCLUSION

The results of the studies presented in this paper indicate that dry skin is generally stiffer than normal skin. Hydration of the skin results in a decrease of both the elastic and loss moduli to the same degree. Tape stripping shows that the outer few layers of the stratum corneum can contribute significantly to the mechanical properties of the stratum corneum, a conclusion also reached by Christensen *et al.* (1). Although the GBE does not measure fundamental mechanical properties of stratum corneum, it can be used to demonstrate that treatment of the skin with agents such as glycerol can result in softer skin. It was also observed that stratum corneum stiffness increases as the measurement site is moved down in position on the lower leg.

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