

Electrometric technique for the *in vivo* assessment of skin dryness and the effect of chronic treatment with a lotion on the water barrier function of dry skin

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Synopsis

Changes in skin capacitance induced by occlusion with a film of anhydrous petrolatum were measured with the aid of a pressure insensitive dry electrode on the forearms of 27 healthy volunteers, most of whom showed signs of superficial skin dryness. In subjects with dry skin the rate of increase in capacitance, which has been determined to signify diffusion of water to the surface of stratum corneum, was higher than in subjects with skin lacking manifestations of dryness. These results appear to support the concept that dry skin has a diminished ability to retain water. The difference could also be explained based on topographical characteristics and the presence of scales in dry skin. Chronic treatment with an ordinary skin lotion rendered dry skin normal-like with respect to its water barrier function. The technique presented here may have wider applications for testing the functional integrity of stratum corneum *in vivo*.

INTRODUCTION

In previous experiments (1) we observed that electrical measurements taken with a dry metal probe before and after occlusion with anhydrous white petrolatum were useful for analyzing the nature of changes in skin hydration induced by a brief solvent exposure. Solvent treatment caused (a) an increase in skin impedance signifying dryness, and (b) after occlusion, a decrease in impedance at a rate faster than that in untreated skin. Both effects would be expected if the solvent reduced the ability of the most superficial layers of the stratum corneum to retain water. A lowering in skin impedance after treatment with petrolatum has been reported by others (2,3), and it is attributed to the known properties of this material to hydrate the skin by reducing transepidermal water loss (4). Based on these facts, we inferred that the effect described in (b) above reflected the rate at which water from insensible perspiration reached and hydrated the surface of stratum corneum. In dry skin this rate is expected to be higher

in conformance with Fick's law of passive diffusion, which when applied to this case implies that the lower the hydration within the surface layers of stratum corneum, the greater the rate at which water from deeper levels diffuses towards such drier regions.

We also attempted to analyze the effects of the respective solvent exposure by employing a wet electrode and evaporimetry. However, in our hands these techniques yielded far less conclusive data on an equivalent number of sites. Therefore, it was considered that the dry electrode measurements, as described in this paper, may constitute an expedient method for investigating phenomena related to the presence of superficial skin dryness.

There is considerable evidence that cosmetically dry skin [a condition categorized in a recent review by Kligman *et al.* (5) as xerosis vulgaris] is associated with higher electrical impedance when measured with dry electrodes (2) and increased rates of transepidermal water diffusion (TEW) as determined by evaporimetric measurements (6). Thus, the impedance and TEW characteristics in cosmetically dry skin show an apparent analogy with those observed in the solvent-dried skin. Based on this analogy, we decided that the technique used by us to observe the effects of solvent treatment may also be applicable to study similar parameters in dry skin. The present investigation was conducted in an attempt to: (a) observe differences between the electrical properties of normal and dry skin; (b) relate the differences to the water barrier characteristics in the two skin types; and (c) assess the effects of chronic treatment with a lotion on the water barrier properties of dry skin.

MATERIALS AND METHODS

INSTRUMENTATION

The test electrode employed in the study was a vacuum controlled metal probe (Bor-Tru Inc., W. Redding, CT) constructed and used as described elsewhere (1). The main characteristics of this electrode are that the pressure exerted by it against the skin can be accurately controlled, and that with brief applications (fifteen to thirty seconds) it does not significantly alter existing levels of skin hydration. The reference electrode, a strip of aluminum foil folded to eliminate sharp edges, is held by the subject between the tongue and the palate. Both the characteristics of the test electrode and the placement of the reference electrode in contact with the oral mucosa (in which the resistance is relatively low and constant) contribute to a high degree of reproducibility, thus solving one of the problems associated with electrical measurements of skin *in vivo*. A Hewlett-Packard® (Model 4262a) capacitance-resistance meter generating 1.5 volts across the terminals and set to a frequency (f) of 100 Hz completed the circuit. The meter was interfaced with a Hewlett-Packard® Model 85 Microcomputer, which was programmed to control the meter and to calculate impedance (Z), phase angle (θ), and capacitive reactance (X_c) from the primary resistance (R) and capacitance (C) values;

$$Z = \sqrt{R^2 + X_c^2}, \quad \theta = \cotan X_c/R \quad \text{and} \quad X_c = 1/(2 \pi fC).$$

The relationship of these parameters to the electrical properties of skin have been reviewed elsewhere (7,8).

INSTRUMENT VALIDATION FOR MEASUREMENTS OF SKIN CAPACITANCE

For reasons which will be discussed below, the data in this report were calculated on the basis of capacitance measurements. Due to the fact that the electrical bridge employed by us was not especially designed for skin research [unlike those used in other laboratories (2,8)], the capacitative nature of the measurements obtained with it had to be verified experimentally. The following procedure was used for that purpose. Six circular areas (3.8 cm² each) located on the inner forearms of one subject were treated with 5 μ l of an aqueous solution containing 1% Triton X 100[®] (a surface active agent) and glycerine in one of the following concentrations expressed as percentages: 1, 2, 3, 4, 5 and 6 (W/W). This treatment has been shown (1) to produce a wide range of relatively stable skin hydration levels. After two hours of equilibration in an air conditioned room (T = 22°C, RH = 55%) with subject idle and the forearms exposed, routine capacitance measurements were taken from the sites as described above. Then, the measurements were repeated with the test leads connected "in parallel" and "in series" with an external reference capacitor (capacitance decade box, Cornell-Dubilier Electronics[®] No. Carolina 27526). Each time before these measurements were taken, the value of the reference capacitor was set to match to the nearest digit those obtained in the routine determinations. The two sets of the assumed skin capacitance readings, one obtained with the instrument alone and the other with the instrument connected to the external reference capacitor, were tested for conformance with summation rules applicable to ideal capacitors (details are given in the results section).

SELECTION OF SUBJECTS WITH NORMAL AND DRY SKIN

The studies were performed during February and March when the incidence of skin dryness is known (10) to be at maximum in the temperate zone. The subjects (n = 27) were healthy male and female volunteers of twenty-one to fifty-seven years of age (mean = 40.5). Twenty individuals claimed to have dry skin on their extremities and used a moisturizer either during the winter or throughout the year; seven regarded their skin as not dry and did not have to use lotions on their extremities. The subjects were instructed to wash their forearms exclusively with Ivory[®] soap daily and to refrain from using any topical products on the respective sites for seven days prior to the study. A last soap and water wash was done on day seven approximately three hours before the experimental steps described below.

SUBJECTIVE EVALUATION OF SKIN DRYNESS AND ELECTROMETRIC EVALUATION OF TRANSEPIDERMAL WATER DIFFUSION

On the day of testing the degree of dryness on the forearms was scored independently by two members of the laboratory staff on a scale of one (= absent or normal) to five (= severe, marked by the presence of large scales and chapping). The scores were based on the degree of scaliness and superficial roughness evaluated visually by touch, and by the subjects' own perception. After completing the evaluation of dryness, three circular areas each of 3.8 cm² were delineated along the longitudinal axis of the inner forearms.

After at least one hour of equilibration in an air conditioned room (T = 22°C, RH = 55%) in which the subjects sat idle with the forearms exposed, capacitance measure-

ments were taken in the center of each area. The test sites were then occluded with a thin film of anhydrous white petrolatum. The occlusive film was produced from approximately 2.5 mg ($\pm 10\%$) of petrolatum dispensed onto the center of a site and spread over the entire area with the tip of a thin, fire polished glass rod. Care was taken to form a continuous film, and not to apply petrolatum outside the test area. The measurements were repeated ten minutes later when relative stabilization of the new capacitance values had occurred. The two sets of capacitance measurements were used to calculate what we designated as "Water Diffusion Index" (WDi) using the formula

$$\text{WDi} = [C_{10} - C_0]/C_0$$

in which C_{10} = capacitance (in Farads) measured at ten minutes after occluding the skin with petrolatum, and C_0 = capacitance before occlusion. In order to observe more closely the changes taking place after occlusion, in two subjects measurements were taken immediately following application of petrolatum and were repeated at short intervals over the ten minute period.

EFFECT OF TREATMENT WITH A LOTION ON THE RATE OF TRANSEPIDERMAL WATER DIFFUSION

Twelve healthy subjects with dry skin were selected for the study using the criteria described above. After a seven day pretest period, which consisted in washing the forearms with Ivory[®] soap and water, the subjects treated one forearm with a skin lotion daily for ten to twelve days. The lotion was a water-in-oil emulsion containing the following ingredients in decreasing order of concentration: water, diisopropyl sebacate, glyceryl monostearate, cetyl alcohol, glycerine, and mineral oil. The contralateral forearms were left untreated. Both arms were washed with soap and water and thoroughly rinsed shortly before each treatment. A last application of lotion was made at the end of the treatment period. Then after approximately five minutes the arms were washed with soap and water, and the WDi was determined in three sites on each arm as described above.

RESULTS AND DISCUSSION

EVIDENCE FOR THE CAPACITATIVE NATURE OF C MEASUREMENTS

Figure 1 shows that C values observed over a wide range of skin hydration levels conformed with summation rules applicable to pure capacitors. This provides evidence for the capacitive nature of the C values reported in this paper. The results also show a linear relationship between C and skin hydration in agreement with observations made by others (2) in skin hydrated by increased environmental humidity.

RELATIONSHIP BETWEEN SUBJECTIVE SKIN DRYNESS AND ELECTROMETRICALLY DETERMINED WDi

Figure 2 shows the correlation between the dryness scores observed by the two evaluators on the forearms of the experimental group. The sub-group, which lacked signs of superficial dryness, was clearly identifiable within the population; all members of the sub-group were scored "1" on the 1-5 scale (coefficient of correlation = 1). The

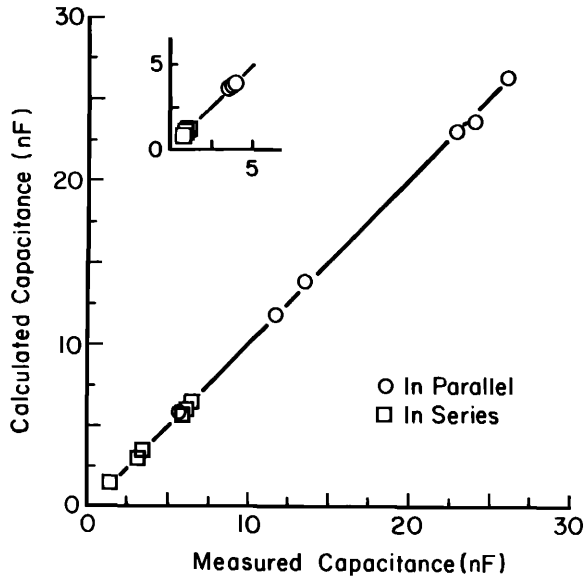


Figure 1. Verification of capacitance measurements in skin after experimentally producing a wide range of hydration levels. The points in the graph were calculated from values obtained (a) with an electrical bridge and (b) with the test leads arranged in series or in parallel with a closely matching reference capacitor. The graph shows that good correlation (coefficient of correlation = 0.98) is seen between the measured values and those obtained by applying summation rules assuming ideal capacitors; $C_t = C_s + C_r$ and $C_t = (C_s \times C_r)/(C_s + C_r)$ for circuits in parallel and in series respectively, in which C_t = total capacitance, C_s = measured skin capacitance and C_r = value of reference capacitor. The insert shows a similar set of determinations in three sites before experimental hydration.

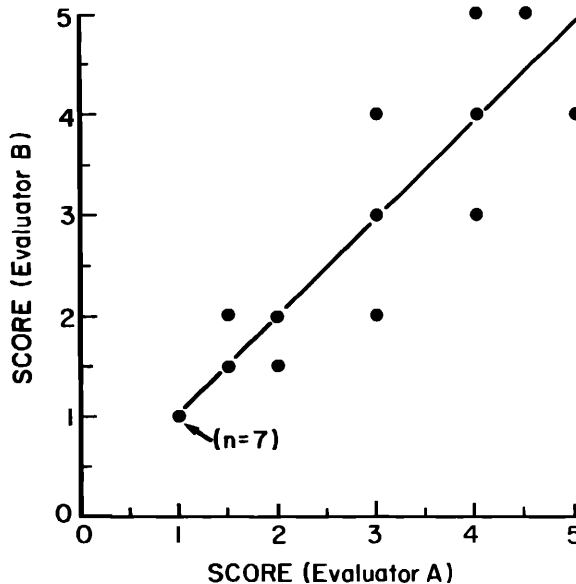


Figure 2. Correlation of subjective dry skin evaluations between evaluators (correlation coefficient = 0.83).

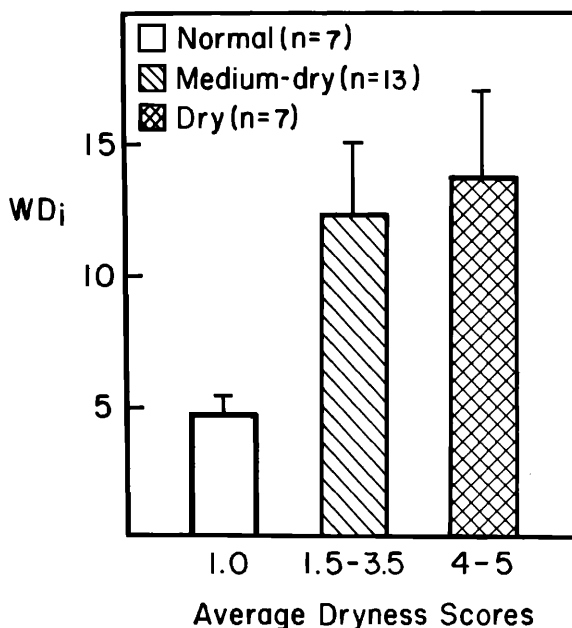


Figure 3. WDi determined electrometrically in skin with different degrees of dryness evaluated subjectively; 1 = absent, 1.5-3.5 = slight to moderate, 4-5 = intense. The bars represent standard error.

scores for the dry skin subjects were less consistent. This was to be expected in view of the great complexity involved in recognizing and quantifying parameters associated with so-called dry skin (5, 10). Despite this, the regression coefficient for the dry group is high enough (0.7) to suggest that different degrees of dryness were detectable within this population.

Figure 3 shows the relationship between the WDi values and the subjective skin dryness. To simplify analysis, the dry skin group is presented as two categories, medium dry and dry, based on the scores ranging from 1.5 to 3.5 and 4 to 5, respectively. The numerical differences in the figure indicate a direct relationship between the WDi values and the degree of superficial skin dryness. However, statistical analyses of data (Table I) show that the differences were significant ($P = 0.05$) only when comparing the normal group to either the medium dry or dry group.

A typical pattern of C readings obtained from two subjects, one of whom had dry skin, is illustrated in Figure 4. This shows that occlusion causes a gradual increase in capacitance associated with a drop in impedance. Immediately following the occlusion with petrolatum (Figure 4 insert) a temporary increase in Z and decrease in C can be

Table I
Statistical Comparison Between Dry, Medium Dry, and Normal Skin Evaluated Electrometrically

Comparison	Geometric Mean Ratio of WDi Values	P Value	95% Confidence Interval
Dry/Normal	2.47	.016	1.20, 5.08
Medium/Normal	1.97	.036	1.05, 3.71
Dry/Medium	1.25	.528	0.61, 2.57

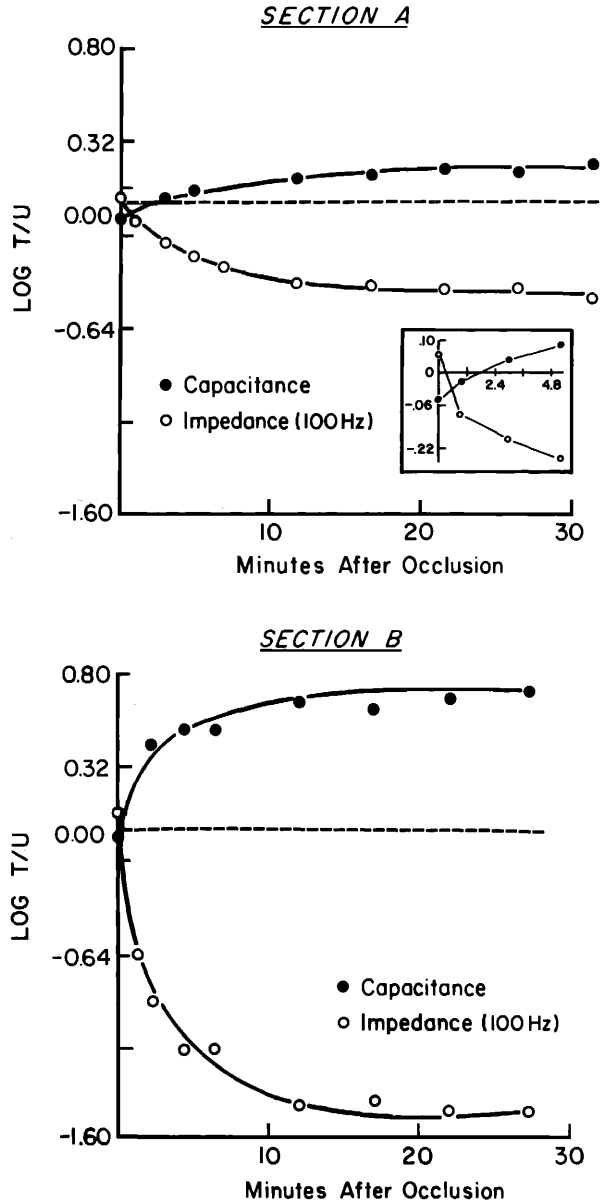


Figure 4. Changes in skin capacitance and impedance measured in normal (A) and dry skin (B) after occlusion with a thin film of white petrolatum. T = petrolatum treated, U = pre-test control measurement.

measured. The latter effects are consistent with the low dielectric constant of this occlusive material. Both the increase in C and the decrease in Z which are observed after a brief time following occlusion have been interpreted (1,2,3) as being related to an increase in skin surface hydration.

The increase in C with hydration (which may appear paradoxical) can be explained based on experiments of Clar [cited in (7)] who found that the dielectric constant (K) of

dry stratum corneum *in vitro* changes with hydration from 2-3 to about 600. Kraning *et al.* (13) also report an increase in K after hydration of skin *in vivo*. The direct relationship between C and K can be observed from the following simplified equation:

$$C = KA/[4\pi D(9 \times 10^{11})]\text{Farads,}$$

in which A is the plate area, D is the difference between plates and 9×10^{11} is a constant.

It has been reported by several investigators (1,2,3) that C and the reciprocal of R correlate closely when measuring superficial skin hydration. On this basis it would appear that either C or R and Z or θ which can be calculated from the former two (refer to methods section) would be equally suitable for determinations of WDi as described herein. However, for several reasons the main results in this report are based on C measurements.

Work in this laboratory (manuscript in preparation) shows that the signal to noise ratio is higher for C than for R when measuring changes in the hydration of the corneal matrix. We also observe that along with the higher sensitivity, C is less dependent on phenomena related to skin galvanic response (SGR) induced by the activation of sweat glands. The latter can constitute a source of error; consequently, its effects on the measurements must be reduced as much as possible (9,11). An additional reason for employing C measurements is based on work by Clausen, *et al.* (14). Their data show

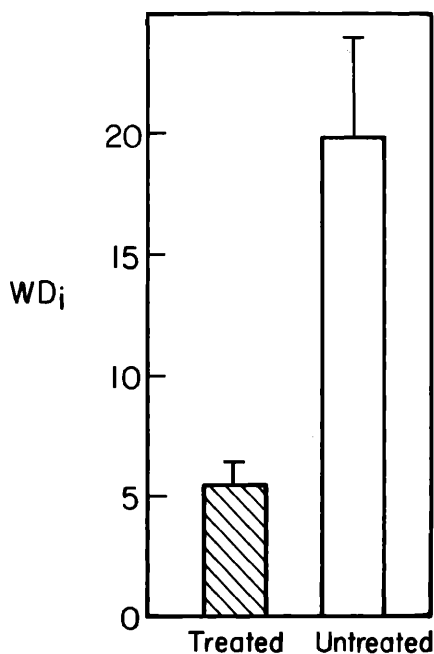


Figure 5. The effect of chronic treatment with a skin lotion on the WDi of dry skin. Note that before the measurements were taken the sites were thoroughly washed with soap and water and allowed to equilibrate with the environment. The bars represent standard error.

that C is a more sensitive indicator of the size of the effective area under the electrode particularly when R is very low. Since one of the objectives of the present work was to observe differences between normal and dry skin in which, due to topographical characteristics, the ratio of nominal to effective area is different (15), C appeared to be the logical parameter of choice.

The properties of stratum corneum with respect to its water barrier function have both medical (2) and cosmetic (12) implications. The technique described in this paper is useful for demonstrating that skin which manifests signs of superficial dryness permits water to diffuse to the exterior at a higher rate than does normal skin. Similar but somewhat less conclusive results have been reported by Leveque *et al.* (6) based on measurements of transepidermal water loss. The increased rate of water diffusion detected at the surface of dry skin by means of C measurements could be accounted for by one or a combination of the following factors:

- (a) A higher ratio of actual to apparent surface area known to exist in dry skin (15), possibly in relation to an error of desquamation (5).
- (b) A partial lack in the ability of the most superficial layers of stratum corneum to prevent loss of water (which with this technique is measured as it accumulates at the skin-petrolatum interface).
- (c) An increase in the permeability of stratum corneum perhaps throughout its entire thickness.

More experimental data would be needed in order to analyze the contributions of these factors to the observed differences.

EFFECT OF TREATMENT WITH A SKIN LOTION ON THE TRANSEPIDERMAL WATER DIFFUSION OF DRY SKIN

Figure 5 shows the effect of treatment with lotion on the WDi values of dry skin. A comparison between the values presented in this figure and those given in Figure 3 reveals that the WDi values in dry skin after treatment with lotion are very close to those measured in normal skin. In addition to a lower WDi, the treated sites have a slightly higher capacitance ($P = 0.01$) than the control sites (Figure 6). This indicates greater hydration of the stratum corneum. Furthermore, after occlusion with petrolatum (Figure 6), the rate at which water diffuses to the skin surface is lower in the treated than in the untreated sites ($P = 0.005$). The two effects (higher initial levels of hydration and decreased rate of hydration with occlusion in the treated sites) are consistent with one another and, although not measured with the same sensitivity, demonstrate a favorable change in the water barrier properties of stratum corneum.

Several studies suggested to us indirectly that chronic occlusion with a moisturizing lotion may result in an improvement of the water barrier function of stratum corneum, and that we may be able to observe the effect using WDi measurements. Studies by Kligman (10) show that treatment of xerotic skin with petrolatum produced a favorable and lasting change in the skin topography. Other experiments (16) appear to demonstrate a cause-effect relation between chronic treatment with a lotion and an improvement in the water sorption properties of stratum corneum. More recently, transmission electron micrographs revealed (17) that regular use of a lotion resulted in

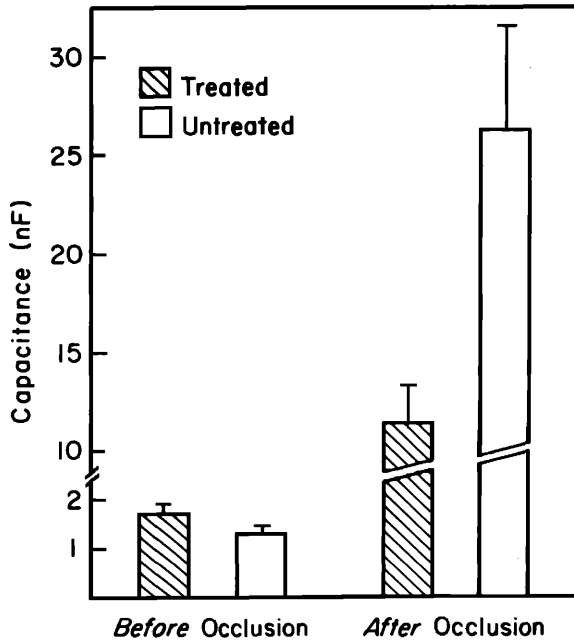


Figure 6. Capacitance measurements taken from dry skin after chronic treatment with a skin lotion; T = treated sites, U = untreated control sites. The bars represent standard error.

thickening of corneocytes located at levels as deep as those adjacent to the granular layer. The effect was attributed by the authors to swelling caused by higher levels of hydration.

The technique used in the present study allowed us to observe a sizable decrease in the rate of water diffusion in dry skin chronically treated with a lotion, in agreement with the effects observed by others as described above. A possible mechanism accounting for the effect of the treatment is the formation of a substantive lipid barrier within the most superficial layers of stratum corneum. This possibility is supported by experiments which show that lipidic compounds, some of which are commonly used in skin lotions, are substantive to the stratum corneum (18), and they produce a positive effect on both the diffusional resistance (19,20) and the electrical capacitance of skin (1,3).

CONCLUSION

This report describes a sensitive method for measuring relative rates of water diffusion at the surface of the skin. The rate is indicated by changes in electrical capacitance as endogenous water accumulates in the outermost layers of the skin subsequent to occlusion with a thin layer of petrolatum.

The diffusion rate is higher in subjects with dry skin than in those with normal skin.

Application of a moisturizing lotion containing glycerine and mineral oil restored the skin to a normal-like condition with respect to its water-barrier function.

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