Electrical Measurement of the Water Content of the Stratum Corneum In vivo and In vitro under Various Conditions: Comparison between Skin Surface Hygrometer and Corneometer in Evaluation of the Skin Surface Hydration State

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Two commercially available electrical instruments which evaluate the hydration state of the skin surface were compared in in vitro and in vivo experiments. The skin surface hygrometer (Skicon-200) employs high-frequency conductance, whereas the corneometer (CM 420, CM 820) uses electrical capacitance to determine the level of hydration. In a simulation model of in vivo stratum corneum (SC), the high frequency conductance device showed a much closer correlation with the hydration state of the surface SC (r = 0.99) than the capacitance device (r = 0.79), suggesting that the former can accurately assess the hydration dynamics of SC, particularly that due to the accumulation of easily releasable secondary bound water and free water. Both devices were insensitive to changes of hydration taking place in deeper viable skin tissues, e.g. the accumulated tissue fluids in suction blisters. Although the capacitance device correlated poorly with the hydration dynamics in normal SC, its sensitivity to changes occurring in extremely dry skin, such as scaly psoriatic lesions, suggests its measurement characteristics at an extremely low state of hydration, consisting of mostly bound water, such as noted in pathologic SC. Key words: Skin conductance; Skin capacitance.

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The function of stratum corneum (SC) is to protect the body from desiccation and invasion by various kinds of external attacks. In addition, it binds water and thereby maintains smoothness and flexibility, so that body movements do not result in cracks or fissures on our skin surface. In recent years, non-invasive electrical devices have become commercially available, two of which are widely used for quantitative assessment of SC hydration kinetics, i.e. the skin surface hygrometer (Skicon-200) (SSH) and the corneometer (CM 420) (1, 2). The latter is considered able to depict changes of hydration much deeper into the skin than the former (2). Although their practical usefulness for such a purpose is well acknowledged, the relationship between the parameters obtained and actual water content in SC has not been fully investigated in vitro, because of the complexity of the distribution of water in SC in vivo. The level of hydration in SC is not uniform but, instead, progressively decreases from the deepest layer that faces the wet living epidermal tissue to the superficial portion that is exposed to the dry ambient atmosphere (1). Recently we devised a simple simulation model of in vivo SC which had such a water gradient and succeeded in demonstrating that the

skin conductance for high frequency current measured with the SSH correlated well with the actual water content of the superficial portion of SC as well as with that of the whole SC (3). As regards the corneometer, despite the fact that it is frequently used for evaluation of the hydration state of the skin surface, no study has compared the parameters obtained with this device and the actual water content of the superficial portion of SC.

The purpose of this study was to examine the correlation between the actual water content in a simulation model of in vivo SC and recorded electrometric data obtained by the two instruments for measurement mentioned above. A comparison of the characteristics of the two instruments was also made in vivo under various conditions in order to determine whether the SSH and the corneometer can be used to measure changes of hydration occurring at different levels of the SC.

MATERIALS AND METHODS

In vitro measurements

Sheet of stratum corneum. We obtained $200~\rm cm^2$ of full thickness skin from the extensor surface of the amputated thigh of a 17-year old girl. The epidermal sheet was separated by placing the skin in water at $60^{\circ}\rm C$ for $30~\rm s$. The sheet was then dipped into a solution of $1\times10^{-4}\rm \%$ trypsin in 5% aqueous sodium bicarbonate. After incubation at $37^{\circ}\rm C$ for $18~\rm h$, the viable epidermis was scraped away. The remaining sheet of SC was then rinsed in distilled water for $1~\rm h$ with one change of water. It was stored in a desiccator over silica gel. This isolated sheet of SC was soft and smooth and retained the water barrier function as well as the ability to hold water (4).

Simulation model of in vivo stratum corneum. As previously reported (3), we placed 5 sheets of filter paper (2.8 cm \times 2.8 cm, TOYO Roshi Co., LTD. Japan) saturated with 20 ml of phosphate-buffered saline on a slide glass. A sheet of SC (3.0 cm \times 3.0 cm) was placed on the filter paper. The free edges of the SC sheet were sealed to the slide glass with vinyl adhesive tape.

Relative humidity chamber. Relative humidities of 33%, 70%, 75%, 90% and 97% were obtained by placing different concentrations of saturated salt solutions at the bottom of desiccation chambers at 20°C (5). Measurements were made after equilibrium was reached, for which 2 h was enough; however, we waited generally for 24 h. Measurements of the same sheet were made at progressively higher relative humidities.

Skin surface hygrometer. The SSH (Skicon-200, IBS Co, Hamamatsu, Japan) was used to measure the conductance by SC. The SSH uses a high-frequency electric current of 3.5 MHz, employing a new sensitive probe consisting of an outer cylindrical electrode, 4 mm in diameter, and a central one, 2 mm in diameter.

Corneometer. The corneometer (CM 420, CM 820, Courage and Kha-

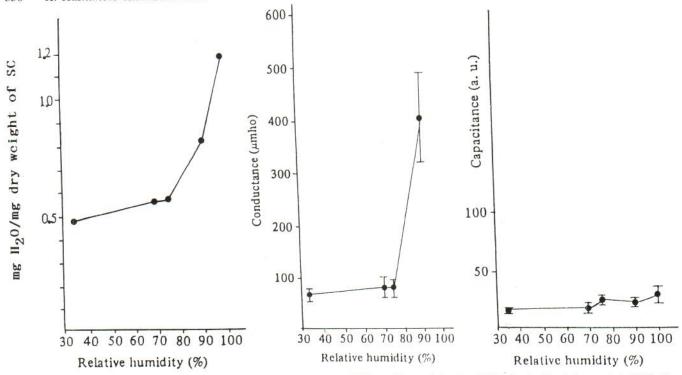


Fig. 1. Relationship between electrical parameters and water content of SC in various relative humidities in the simulation model of SC. Bars represent standard deviations. (a) Water content by weight determination. (b) Skin surface hygrometer measurement. (c) Corneometer measurement.

zaka, Köln, Germany) registers the electrical capacitance of the skin surface.

Gravimetrical determination of water content in the stratum corneum. The dry weight of each SC sheet containing about 5% primary bound water as reported before (3), was measured after 48 h storage in a chamber containing silica gel. Gravimetrical measurements were performed again immediately after conductance and capacitance measurements. After removal from the model, the SC sheets were weighed as soon as possible to avoid changes in water content. The gravimetrically determined water content of the SC sheet was calculated as follows (6):

 $mg H_2O/mg SC dry weight = (wet weight-dry weight)/dry weight.$

In vivo measurements

Three healthy volunteers (2 women and 1 man), aged between 22 and 35 years, and 2 patients with severe psoriasis vulgaris (1 woman and 1 man), both 65 years old, participated in the study after giving informed consent. All measurements were performed in the condition of 18–20°C and 35–45% relative humidity (RH).

Water sorption—desorption test. The water sorption—desorption test was performed as previously described (7). Briefly it consists of electromeasurements before and after application of a droplet of water on the skin for 10 s, to obtain data on the hydroscopic property of the skin surface, and serial measurements at intervals of 30 s for 2 min to evaluate the water-holding capacity by calculating rapidly gained and lost bound water in SC.

Suction blister formation. Blisters were produced on the skin of the extensor surface of the forearm using a hollow 2 ml disposable syringe (Jintan Pharmacological Co., Tokyo, Japan) from which the piston had been removed. The broad and flat end was applied to the skin surface, and negative pressure of 250 mmHg was applied to the nozzle end of the syringe with a rubber tube attached to a vacuum pump belonging to our hospital (8).

Occlusion. The skin surface was occluded by a sheet of polyethylene film for a designated time period, and measurements were performed after removal of the occlusive film.

Statistics. At least 3 measurements were performed to obtain a mean value for each of the experiments except for gravimetrical determination of water content in SC. The level of significance was calculated by Student's *t*-test for paired comparisons.

RESULT

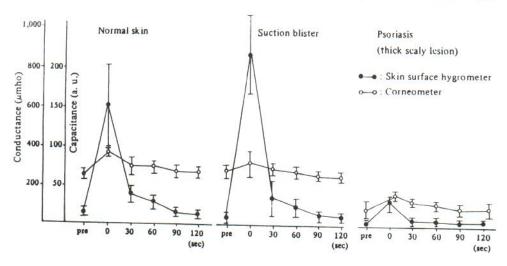
In vitro measurements

Measurements at various relative humidities. Water content in the SC sheet placed in the simulation model of in vivo SC was about 33% at 30% RH, which increased gradually until a dramatic increase occurred when the RH was above 75%. High-frequency conductance and low-frequency capacitance increased gradually in relation to RH up to 75% RH. Thereafter, in contrast to the capacitance which showed a very small increase, the high-frequency conductance increased sharply (Fig. 1). The correlation coefficient between the actual water content by weight determination and conductance measured with the SSH was 0.99 (p < 0.01). In contrast, the correlation coefficient with capacitance recorded with the corneometer was 0.79 (p < 0.05).

In vivo measurements

Water sorption—desorption test. Conductance values measured with the SSH on the skin surface increased sharply after absorption of water for 10 s. They decreased gradually, approximating an exponential curve. The pattern of the curves obtained on the top of a suction blister was similar to those on normal skin. Conductance was extremely low on the thick

Fig. 2. In vivo water sorptiondesorption test measured with SSH and corneometer (18°C, RH 37%). Bars represent standard deviations.



scaly lesions of psoriasis vulgaris even after absorption of water. By contrast the curves based on capacitance as determined by the corneometer showed much less change whether on normal skin or on the top of suction blisters, as was also the case with psoriatic lesions. But in general capacitance values were much lower on psoriatic lesions than on normal skin (Fig. 2).

Effect of occlusion. When the normal skin surface was occluded by a sheet of polyethylene film, conductance values obtained with the SSH increased steadily, whereas capacitance measured with the corneometer showed only a small change (Fig. 3).

Influence of serial stripping of the SC. Serial tape-stripping of the SC produced a progressive increase in conductance. The increase in capacitance observed was again much smaller than that noted in conductance (Fig. 4).

Correlation between skin conductance and capacitance. When conductance and capacitance were measured on various sites

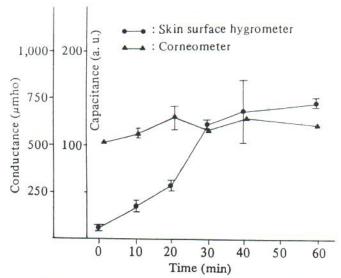


Fig. 3. Effect of occlusion on the electrical parameters in vivo on the skin surface (18°C, RH 37%). Bars represent standard deviations.

of involved and uninvolved skin of patients with psoriasis, there was a positive correlation between the values obtained $(r=0.60,\ p<0.01,\ {\rm Fig.}\ 5)$ although the X-Y intercept was clearly different from 0. Capacitance tended to show a wider range of distribution than high-frequency conductance when measured on dry skin such as psoriatic skin.

DISCUSSION

The present study showed that the two instruments selected for comparison, i.e. the SSH and the corneometer, had rather different characteristics in various in vitro and in vivo experiments. Previously we reported that, in a simulation model of in vivo SC, high-frequency conductance values correlated well with the actual water content in the uppermost portion of SC, as well as with the water content of the whole SC (3). In this study, we found that the water content determined gravimetrically correlated far better with the conductance values

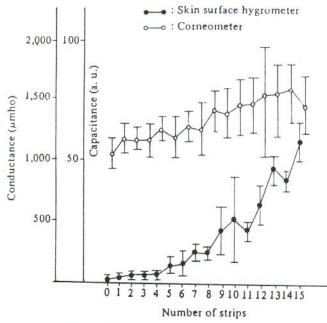


Fig. 4. Influence of serial stripping of stratum corneum on the electrical parameters in vivo (18 $^{\circ}$ C, RH 37 $^{\circ}$). Bars represent standard deviations.

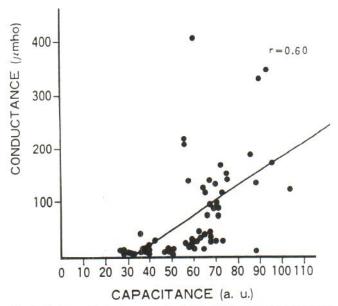


Fig. 5. Relationship between conductance and capacitance measured in scaly lesions of various grades of severity and in the uninvolved skin of 2 patients with psoriasis (r = 0.60; p < 0.01).

measured with the SSH than the capacitance values obtained by the corneometer. Thus, the SSH seems to be better suited both for the evaluation of the efficacy of topical agents, such as moisturizers, that enhance the hydration levels of SC (9) and the performance of the water sorption–desorption test (7). However, the great sensitivity of this instrument at a highly hydrated state and rather large variations in the data obtained in such a situation suggest that great care should be taken for its application. It should be applied in the same manner as much as possible to obtain reproducible results in the measurements

The SSH was once thought to measure the water content of only the very superficial part of SC (1). In contrast, the corneometer has been considered to depict changes in hydration down to a depth of about 0.1 mm (2). We began this study expecting to make a more thorough analysis of the hydration state at different levels in SC by using the SSH and the corneometer together. We think that a subcorneal bulla is the best model for such a purpose, but in practice, it was difficult to produce experimentally a tough subcorneal blister that could withstand the repeated measurements. Hence, we produced a suction blister in vivo to increase the water content at a depth of about 0.1 mm, assuming that the thickness of the epidermis is between 0.075 to 0.15 mm (10). The capacitance values thus obtained on the top of the suction blister with the corneometer were not higher at all than those from normal skin. This was also the case with the measurements made after serial tapestrippings of SC which were performed to reach progressively deeper and wetter portions of SC. The concomitant increase in capacitance was much smaller than that noted with high-frequency conductance. Moreover, the recorded capacitance values always showed a disappointingly small increase even after occlusion of the skin surface, which allows deeper as well as superficial portions of SC to absorb water. This leads to a very small variation in data obtained with the corneometer under

these experimental conditions. However, all of these in vivo findings suggest that, although the capacitance values obtained with the corneometer may reflect the hydration state of the upper portion of SC, they do so in a much less sensitive way than the conductance values obtained with the SSH.

The fact that there was only a small increase in capacitance when the drier and more superficial parts of SC were stripped away or when the skin surface was occluded to hydrate the upper portions of SC might still be seen as an indication that the corneometer is more sensitive to changes in the deeper parts of SC than the SSH, because the capacitance values would be affected to a minor degree by the changes in the superficial parts as compared to those in the deeper portions, whose hydration levels are thought to remain stable even during these experiments. However, the magnitude of the changes observed does not warrant that the corneometer is practically applicable for the evaluation of the hydration state at the deeper portions of SC.

To plasticize SC, water binds molecularly and this bound water is about 30% of dry SC weight in normal SC samples (11, 12). There are two separable bound water fractions, i.e. readily releasable water and more tightly bound water. When the amount of accumulated water exceeds the upper limit of the bound water, water exists as bulk or "free" water. The rapid and remarkable increase in water content that occurs instantaneously in normal SC just after absorption of water in a water sorption-desorption test is thought to mainly reflect changes in the amount of easily releasable secondary bound water and free water. Thus, the high frequency conductance measured with the SSH seems to be sensitive to the changes in the secondary bound water and free water in the superficial portion of SC. In contrast, the capacitance measured with the corneometer is too insensitive to such hydration dynamics noted in normal SC (13, 14).

Despite this poor sensitivity to the hydration process taking place in the SC of normal skin, the capacitance method seems to exerts its practical usefulness in the measurements of extremely dry scaly skin, because the difference in capacitance between normal skin and dry scaly psoriatic lesional skin appears to be unproportionately large in the values obtained, as reported by Van Neste (15). A possible explanation for such responsiveness to poorly hydrated SC is that the optimal range of water content for the corneometer measurement is much lower than that for SSH, i.e. possibly in a range consisting of mostly bound water (11, 12). The large measuring area in an extremely dry state is one of the advantages of this instrument. The surface hydration state of a SC sheet mounted on the simulation model does not differ greatly whether it is placed at 0% RH or at 30% RH, as long as the SC sheet is prepared from normal skin (3). Thus, for elucidation of the correlation between skin capacitance and water content in dry SC, we need a simulation model that uses pathologic SC instead of normal SC. However, we have been unable to obtain reliable data so far using such a model constructed with flakes of scales obtained from psoriatic lesions (unpublished data).

In conclusion, the present study provides evidence that the high-frequency method accurately assesses the hydration state of SC in normal skin or in hydrated skin; the correlation of conductance values with the actual water content in normal SC is much higher than that of capacitance values obtained with the corneometer. In contrast, the wide measuring area of the latter in a poorly hydrated state seems to provide an advantage for the measurements made in such a situation. Comparative data obtained in a substantial number of subjects under various clinical situations are required to delineate these characteristics of the two methods more clearly. For demonstration of the relationship between the hydration state of SC and parameters obtained with the corneometer, we need a simulation model of in vivo dry pathologic SC.

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