Water Sorption-desorption Test of the Skin in Vivo for Functional Assessment of the Stratum Corneum

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Based on the evidence from our previous studies that we can evaluate the hydration state of the skin surface quickly and quantitatively in terms of conductance to the high frequency electric current of 3.5 MHz, we have established a simple in vivo function test that furnishes information on the hygroscopic property and water-holding capacity of the stratum corneum in a few minutes. The test procedure consists of electromeasurements before and after application of a droplet of water on the skin for 10 seconds to obtain data on the hygroscopic property of the skin surface and later serial measurements at an interval of 30 seconds for 2 min to evaluate the water-holding capacity. Under usual ambient conditions normal skin surface showed a high rise in conductance just after application of water, which was followed by a rapid fall-off within 30 seconds, thereafter by gradual return to the prehydration levels by 2 min.

By this method we have demonstrated that (i) the superficial horny layer of normal skin is much less hygroscopic and less capable of holding water than the corresponding deeper portions and that (ii) scaly skin shows functional defects in both hygroscopicity and water-holding capacity, between which the former normalizes much faster than the latter.

The primary function of the epidermis is to produce a protective sheath, the stratum corneum, that protects the body from dessication and invasion of various kinds of external attacks [1]. For this purpose we need a tough yet thin and flexible barrier membrane. The water content of the stratum corneum plays an important role in providing the skin surface with suppleness. In vitro studies with excised pieces of stratum corneum showed that they are flexible as long as they contain more than 10% of water [2]. However, such a situation occurs only in excised pieces of stratum corneum. Since in vivo the lowermost portion of the stratum corneum which directly faces the water-saturated viable epidermis is always well hydrated, while the upper portion is much drier because it is exposed to ambient air. Since the water content in the superficial portion of the stratum corneum fluctuates depending on the ambient humidity, even normal skin surface may crack finely in areas prone to flexing and stretching to develop the clinical feature of "dry skin" or chapping when the thickness of the portion that contains water less than 10% reaches a certain depth. In contrast, even under normal environmental conditions pathological horny layer which is defective in barrier function may readily lose water to develop a scaly clinical appearance.

Until recently we have lacked methodologies that can quickly furnish quantitative information on the skin surface hydration state. Our earlier studies demonstrated that we can evaluate it rapidly and quantitatively without changing the surface properties of the skin by employing an instrument that measures the conductance, i.e., the reciprocal of resistance, to the high frequency current of 3.5 MHz [3]. By this method it becomes possible to reveal that the water content of the stratum corneum progressively increases from the surface to deeper portions and that the normal skin surface absorbs water instantaneously and desorbs it quickly. Although with this method we can appreciate that the water content of scaly skin is very low as compared to the adjacent normal skin, such a difference tends to become negligible in a dry ambient condition since the water content in the superficial portion of the normal stratum corneum also decreases markedly under such conditions. These findings prompted our attempt to improve the methodology to provide much more extensive, functional data on the hydration and dehydration kinetics of the stratum corneum in vivo.

In this paper we describe a water sorption-desorption test of the skin that furnishes information of the hygroscopicity and water-holding capacity of the surface horny layer in a short time. By this test it has been disclosed that scaly skin lacks both of these capabilities. In this context we use the following words under the definitions given below, i.e., hygroscopicity denotes the property of stratum corneum to take up (=sorb) water and water-holding capacity, the ability of stratum corneum to retain water opposing a dehydration process (=desorption).

MATERIALS AND METHODS

Instrument
A detailed description of the measuring principle was made previously [3]. A new model of the instrument that was made by IBS Inc* was used. It automatically prints every reading of conductance in terms of $\mu$F and capacitance in $\mu$F on an attached paper 3 seconds after application of a probe on the skin. The printing apparatus works only when there is a change of more than 5 $\mu$F in conductance; thus, in case with very low conductance values reading should be made directly from a digital recorder.

Throughout this study we have used the same probe as that used in the previous study [3]. We can increase sensitivity of measurement by choosing a probe with a central electrode larger than 2 mm in diameter, whose extreme sensitivity, however, becomes a drawback for routine measurement.

Since the hydration state of the stratum corneum increases gradually from the surface to the lowermost portion within a thickness of about 20 $\mu$, even a small manual pressure on the probe increases the observed value. Therefore, to obtain reproducible results, the flexible cable that connects the probe with the recording body was held by the fingers of an examiner at about 10 cm distance from the probe, which was gently lowered on a test area to rest only with its own weight of 80 g on the skin without applying any additional manual pressure.

Subjects
Hospital personnel, medical students, and out- and in-patients seen at the Department of Dermatology, Hamamatsu University School of Medicine took part in the study.

Test Procedure
The edge of the test area at least 1 cm in diameter was marked on

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the skin with a ball-point pen to avoid missing the site in repeat measurements since it became impossible to find the site of artificial hydration after blotting.

At first a conductance-value of the test area was measured to obtain a prehydration level. Then, a drop of distilled water was placed over the test area for 10 seconds. Immediately after blotting the site with a pad of gauze, a measurement was made and was repeated at an interval of 30 seconds for 2 min. For artificial hydration other aqueous solutions such as saline instead of distilled water can be used since the results are not greatly affected by the presence of electrolytes but only by exogenous supply of water as reported before [3].

RESULTS

Measurements on Normal Skin

As reported before [3], normal skin shows a marked increase in conductance to the high frequency current just after blotting of applied water. Most of this increase is lost within 30 seconds under usual ambient conditions, followed by a gradual return to a prehydration level. Thus, pilot experiments were carried out on neighboring areas of the flexor aspects of the forearms in 6 healthy subjects aged between 30 and 41 yr by changing the duration of application time of distilled water from 10 to 60 seconds at a room temperature of 24°C and relative humidity of 50%.

As shown in Fig 1, the desorption curves resembled each other. However, when a water-droplet was applied on the skin longer than 10 seconds, the increased conductance-values took longer than 2 min to return to the preapplication level. Therefore, we have chosen 10 seconds as an application time of distilled water for the practical reason that the whole test procedure can be finished in just over 2 min.

Comparison between the Surface and Deeper Portion of the Stratum Corneum

The test was performed on the same area of the flexor surface of the forearms of 11 normal subjects aged from 25 to 55 yr before and after partial stripping of the horny layer to assess whether there is any functional difference between the superficial and deeper portion of the horny layer.

After finishing the first test on the normal skin surface, the stratum corneum of the site was stripped away with an adhesive tape 10 times. Since the inside of the stratum corneum is more hydrated than the surface, an interval of 3 min was allowed before the start of the next measurement to permit the increased conductance-values to settle at a certain level (Fig 2).

![Fig 1. Changes in conductance-value after application of distilled water for various periods ranging from 10 to 60 seconds. Bar represents standard deviation.](image1)

The results of the second test showed that there was a prominent increase in conductance immediately after blotting of a droplet of water, pointing out much higher hygroscopic property of the stratum corneum at deeper portions than at the surface. This increase in conductance returned to the prehydration level after 60 seconds, which was somewhat higher than that obtained on the skin surface, but the difference was not significant at the 5% level.

Measurements on Scaly Skin

Fig 3 shows the summarized results of the test performed in 97 randomly selected patients with various kinds of scaly dermatoses. Solid circles represent a mean value from scaly lesions and open circles that from adjacent normal skin. All the parameters denoting (1) prehydration level, (2) hygroscopic property, and (3) water-holding capacity are decreased in scaly lesions.

![Fig 2. Water sorption-desorption test on normal skin surface and that repeated 3 min after adhesive tape-stripping of the horny layer 10 times.](image2)

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Measurements on Scaly Skin

Fig 3 shows the summarized results of the test performed in 97 randomly selected patients with various kinds of scaly dermatoses. They consisted of 50 patients with psoriasis, 39 with eczematous dermatitis, 3 with tinea corporis, and one each with subcorneal pustular dermatosis, keratosis follicularis, pityriasis...
Lesions, As compared to diffusely scaly lesions. Lesions with thick scales are almost zero regardless of the background disorders and the difference between scaly lesions and normal skin became much more apparent at the reading taken immediately after blotting of water, indicating lower hygroscopic property of scales. On scaly skin such slightly increased conductance-values quickly returned to zero, reflecting defective water-holding capacity.

**Clinical Correlation in Scaly Lesions**

The above mentioned impaired functional parameters in scaly lesions were further analyzed in correlation with the clinical severity, selecting 2 representative dermatoses, i.e., psoriasis and eczematous dermatitis.

Psoriatic lesions were arbitrarily grouped into 2 categories, i.e., thick scaly lesions that showed the presence of thick silvery scales and thin scaly ones that were accompanied by only thin scales. The readings made on those with thick scales were always near zero except for that made just after blotting out of water when a trace increase in conductance was observed, whereas the thin scaly lesions showed a moderate increase at this reading although others were still almost zero. (Fig 4, a and b).

Lesions of chronic eczematous dermatitis were also grossly divided into 2 groups. Since in this case scaling was not so prominent as in psoriasis, they were classified as diffusely scaly lesions when scales were observed all over the test area and as partially scaly lesions if there were only scattered fine scales. As shown in Fig 5, again there was a good correlation between the clinical severity of scaling and results, particularly those obtained just after blotting of applied water.

Such a correlation between the clinical evaluation of scaling and measured results was also observed in the same lesions if they were followed in the course of successful treatment. Fig 6 shows a representative example noticed in a 27-yr-old patient with psoriasis in whom topical 8-methoxypsoralen application followed by irradiation of long ultraviolet light (topical PUVA) showed a beneficial effect within 7 weeks. As the lesion began to improve after 2 weeks of treatment, the hygroscopic property of the lesional horny layer at first began to increase. This trend became more apparent in the following weeks. At 7 weeks after initiation of treatment when the lesion completely regressed, all the parameters reached the same levels as those on the adjacent normal skin.

**DISCUSSION**

The results in the present study show that by employing the water sorption-desorption test we can perform much better functional analysis of the stratum corneum in vivo than the simple measurement of the skin surface hydration state, since we can obtain important parameters such as (i) in vivo hydration state, (ii) hygroscopic property and (iii) water-holding capacity of the stratum corneum in a very short period of time. If the test area is wide enough, we can increase the precision of the measurements by repeating the test procedure several times in neighboring areas.

**In vitro** kinetic studies on water sorption-desorption by human stratum corneum gave information on the existence of at least 2 species of water in stratum corneum [4]; rapidly sorbed-desorbed water to the extent of 0.5 mg/mg stratum corneum is a "bound water" which is an energetically bound pool of water, whereas slowly sorbed-desorbed "free water" is a nonenergetically bound pool of a bulk liquid water which is absorbed up to 12 times dry weight of stratum corneum until the tissue begins to break down mechanically. It is reasonable to presume that the measurements of water content in stratum corneum in our water sorption-desorption test are chiefly concerned with the amount of the rapidly gained and lost "bound water" in stratum corneum. We have found that the stratum corneum of the superficial portion is less hygroscopic and somewhat less capable of binding water than that of the deeper portions. This seems to be due to a deficit in small-molecular-weight water-soluble hygroscopic substances that are derived from the viable.
epidermis [5], i.e., so-called "natural moisturizing factor" [6], which may be leached out from the skin surface by washing or by taking baths or showers.

The data presented here demonstrate general agreement in that the stratum corneum of scaly skin is always dry because it is less hygroscopic and markedly less capable of binding water than normal skin. Particularly the defective water-holding capacity seems to play a crucial role; even eczematous lesions with inconspicuous fine scales showed an almost complete lack of this capacity and normalization of the decreased water-holding capacity of scaly lesions took place only at the end of successful treatment, while hygroscopicity of such stratum corneum gradually returned to normal with improvement of the lesions. These findings are probably explained by the fact that normal water-holding capacity requires complete restoration of stratum corneum with intact structure, whereas hygroscopicity of the stratum corneum depends on the presence of the hygroscopic substances of small molecular weight on the superficial portion of the stratum corneum being not covered by such substances as dessicated tissue fluids.

Up to date we have not had in vivo methodologies which are capable of providing quantitative parameters of "dry skin" [7]. We can apply this methodology for assessment of such skin; in fact, some cases of eczematous dermatitis with partially scaly lesions in the present study were those with eczema craquelé which developed from pruritic dry skin occurring in winter. We can also use this method as a rapid in vivo screening test for topical agents such as moisturizers and emollients; by this method we have succeeded in disclosing the moisturizing effect of 10% urea cream on normal skin (unpublished data). Therefore we think that it is applicable to many fields and that it opens a new way for functional analysis of the stratum corneum.

REFERENCES

Announcement

A Hawaiian Dermatology Seminar will be held in Maui, Hawaii, February 1-6, 1983. This is a limited enrollment Course in Dermatology and is oriented primarily to dermatologists involved in patient care. This postgraduate course is sponsored by the Northwestern University, Cleveland Clinic, and University of California (San Francisco) Departments of Dermatology. Speakers include faculty of these departments and guest faculty. The presentations will emphasize diagnosis and treatment of common cutaneous disorders. For registration information and preliminary program, write: Department of Dermatology, Northwestern University Medical School, 303 E. Chicago Ave., Chicago, IL 60611.