Arch Dermatol Res (1991) 283:342–346

Archives of Dermatological Research

In vitro comparison of water-holding capacity of the superficial and deeper layers of the stratum corneum

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Received January 5, 1991

Summary. We measured the electrical conductance at 3.5 MHz of a sheet of normal stratum corneum (SC) mounted with either the upper surface or the lower surface downward in simulated in vivo conditions. In this way, we assessed the water-binding capacity of the upper and lower portions of the horny layer. Measurements were made with the SC model in environments of various relative humidities. Between 30% and 90% relative humidity, the conductance of the upper surface was always significantly higher than that of the lower surface. In contrast no significant difference was observed in transepidermal water loss (TEWL) with the SC sheet placed upwards or downwards. After exposure to lipid extraction with acetone/ether, a significant decrease in conductance and increase in TEWL occurred, particularly in the upper surface. The amount of extractable amino acids was much higher from the middle layer than from the outer layers of the SC, and was lowest from the deepest part of SC. These results, indicating a lower efficiency of the lower surface of the SC for water-binding than the desquamating upper surface, suggest that newly formed immature SC does not have the water-holding capacity of the mid portion of the SC, which is probably the layer with the greatest water holding capacity.

Key words: Amino acids – Barrier function – Horny layer – Hydration – Natural moisturizing factor

The stratum corneum (SC) functions to protect the body from desiccation and to block ingress of harmful substances. Its barrier properties are renowned. In addition, it provides a smooth and flexible surface by binding water so that body movements do not result in cracking or fissuring [7]. When functional parameters of the SC are compared between normal and pathologically scaly skin, there is an inverse correlation between the water barrier function and hydration function of the SC [15]. Serial stripping of the SC in vivo has shown that the level of hydration, measured by the conductance of high frequency electrical current at 3.5 MHz, progressively increases as deeper layers of the SC are exposed [16]. On the other hand, barrier function, assessed by transepidermal water loss (TEWL), shows a gradual decrease [1, 18]. Moreover, an in vivo functional study of the SC by a sorption – desorption test revealed that the water-binding capacity of the middle layers of the SC was greater than outermost layers [17]. Thus, we speculated that the newly produced, deepest region of the SC might be much more hygroscopic than the superficial region. However, in vivo it is impossible to evaluate the water-holding capacity of the deepest portion of the SC because it is continuous with water-saturated viable epidermis and is always hydrated. The upper air-exposed portion is always much drier, especially at low humidities.

The purpose of this study was to compare the actual water-holding capacity of the upper and lower surfaces of the SC in vitro by using an in vivo simulation model. We have previously established that the 3.5 MHz conductance values closely correlate with the actual water content in the superficial portion of the SC and in the whole SC [11].

Materials and methods

Preparation of stratum corneum sheets

We obtained 200 cm^2 of full thickness skin from the extensor surface of the amputated thigh of a 17-year-old woman. The epidermal sheet was separated by placing the skin in water at 60° C for 30 s. The sheet was then dipped into a solution of 1×10^{-4}% trypsin in 5% aqueous sodium bicarbonate. After incubation at 37° C for 18 h, the viable epidermis was scraped away. The sheet was then rinsed in distilled water for 1 h with one change of water [8] then stored in a desiccator over silica gel. Five other SC samples from five other adults were also similarly prepared.

Simulation model

Five sheets of filter paper (1.8 × 1.8 cm, Toyo Roshi Co. Ltd, Japan) saturated with 20 ml phosphate-buffered saline were placed on slide

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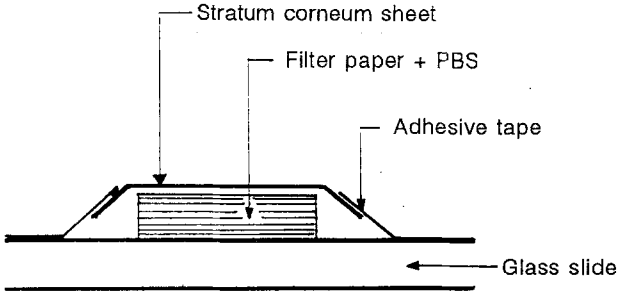

Fig. 1. Schematic diagram of the in vivo simulation model for stratum corneum. An isolated sheet of stratum corneum covers a pad of overlapping filter paper saturated with phosphate buffered saline (PBS). All the free edges of the stratum corneum are sealed to a slide glass with a removable frame of adhesive vinyl tape. The upper surface of the stratum corneum is exposed to the ambient atmosphere

glasses. Sheets of SC (2.0 × 2.0 cm) were placed on the filter paper with either the lower or the upper surface downward. The free edges of the SC sheets were sealed to the slide with adhesive tape [11] (Fig. 1).

Relative humidity chamber

Relative humidities (RH) of 33%, 70%, 75%, 90% and 97% were obtained by placing various saturated salt solutions at the bottom of desiccation chambers [9]. Measurements were made after equilibrium was reached, generally 24 h. Measurements of the same sheet were made at progressively higher RH.

Extraction of lipids

An open-ended cylinder of diameter 2 cm was pressed onto the exposed surface of the SC sheets and acetone/ether (1 : 1) was applied for 5 min to remove the lipids.

Assessment of hydration

Electrical conductance measurements were performed with a skin-surface hygrometer (Model SKICON-200, IBS, Hamamatsu, Japan) [14, 16]. A new probe was used consisting of an outer cylindrical electrode of diameter 4 mm and a central electrode of diameter 2 mm. The sensitivity of this instrument was estimated to be three times greater than a conventional probe.

Water sorption–desorption test

The water sorption–desorption test was performed as previously described [15]. In brief, electromeasurements were made before and after application of a droplet of water onto the skin for 10 s to obtain data on the hygroscopic property of the skin surface, and serial measurements were made at intervals of 30 s for 2 min to estimate the water-holding capacity by calculating the water desorption rate. By this method we were able to determine the amount of rapidly gained and lost bound water in the SC. The term hygroscopicity denotes the property of the SC to take up water, and water-holding capacity the ability of the SC to retain water against dehydration.

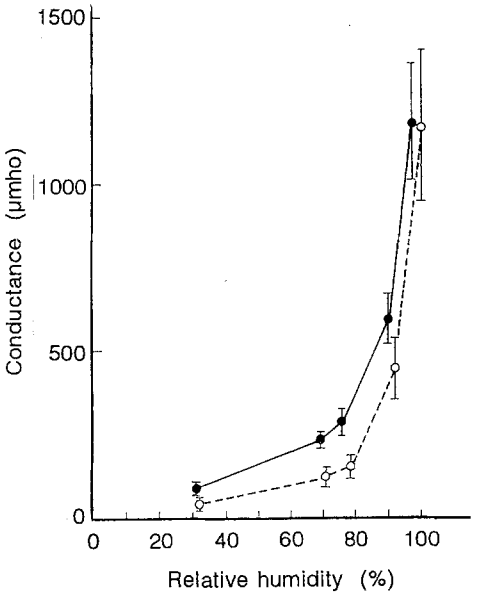

Fig. 2. Relationship between RH of the ambient atmosphere and conductance measured on the upper surface (*solid circles*) or on the lower surface (*open circles*) of a stratum corneum sheet which was mounted with either the upper surface or the lower surface upward. The conductance values were significantly lower ($p < 0.05$) on the lower surface than the upper surface except for those measured at 97% RH

Transepidermal water loss

TEWL was measured with an evaporimeter (ServoMed, Stockholm, Sweden) described by Nilsson [10]. All the measurements were carried out at room temperature (18–20° C) and 35–50% RH.

Determination of amino acid content

A sheet of SC was attached to a slide glass with double-sided Scotch tape. This was then serially stripped with adhesive tape (Cellotape, Nichiban Tokyo, Japan), and the adherent corneocytes were removed from the tape by immersion in toluene, followed by drying. After homogenization in 0.3 N $HClO_4$ in a glass homogenizer and centrifugation, the amino acid content of the supernatant was determined by an amino acid analyser (Hitachi, Tokyo, Japan, model 835). The acid-insoluble precipitate was dissolved in 1 N NaOH, and the content of amino acids expressed as nanomoles per mg of alkaline soluble protein [4]. A control experiment with adhesive tape alone yielded only a negligible amount of amino acids.

Statistics

The level of significance was calculated using Student's *t*-test for paired comparisons.

Results

Measurements at various relative humidities

Conductance measured either for the upper or lower surface of the SC increased gradually with RH up to about 75% RH, and thereafter increased sharply. However, in the range 30–90% RH, the conductance values were significantly lower on the lowest surface of the SC (Fig. 2); only at 97% RH was no difference in conduc-

Table 1. The high frequency conductance and transepidermal water loss (TEWL) measured at 20°C and 75% RH in six samples of stratum corneum sheet mounted with the upper surface or lower surface upward in the in vivo simulation model

Subject no.	Age	Sex	Site	Conductance (μmho)		TEWL (g/m^2 per h)	
				Upper surface	Lower surface	Upper surface	Lower surface
1	17	F	Thigh	277	140	2.7	2.7
2	20	M	Thigh	340	204	8.2	8.6
3	26	F	Buttock	167	74	5.6	5.5
4	62	F	Leg	46	37	2.0	2.6
5	62	F	Thigh	47	11	3.0	2.8
6	70	M	Thigh	54	17	5.4	5.0

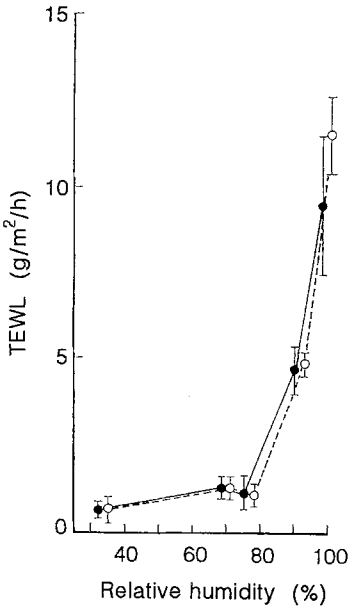

Fig. 3. Relationship between RH and TEWL measured when a sheet of the stratum corneum was placed with the upper surface (*solid circles*) or lower surface (*open circles*) upward. No statistically significant difference was found whichever way up the stratum corneum sheet was placed

tance noted. To verify this finding, we performed the conductance measurement at 70% RH in similarly prepared SC samples from five other individuals. As shown in Table 1 conductance values were always lower in the lower surface of the SC sheet than in the upper. Moreover, SC samples prepared from elderly people showed much lower values than those from young people.

In contrast, TEWL values were the same whether the upper or the lower surface was placed downward (Fig. 3). These findings were again confirmed with other SC samples (Table 1).

The functional parameters measured by the water sorption–desorption test are given in Table 2. There were significant differences at greater relative humidities. Calculated desorption rate constants of the upper surface were significantly lower than those of the lower surface at 69%, 75% and 90% RH. The hygroscopicity of the upper surface was significantly greater than the lower surface only at 33% RH ($p < 0.05$).

Effect of lipid extraction

A significant decrease in conductance occurred after treatment of the surface with acetone/ether. This decrease was much more striking in the upper than the lower surface (Fig. 4) ($p < 0.05$). TEWL also showed a significant increase after delipidization (Fig. 5) ($p < 0.05$).

Amino acid content

With our tape, the lowest layer was reached after 13 strippings. Serial strippings were combined into the following samples: (1) strips 1–3; (2) strips 4–6; (3) strips 7–9; (4) strips 10–12; and (5) strip 13. Samples 1 and 2 comprised loose scales, and samples 3 to 5 apparently comprised monolayers of corneocytes. The amount of amino acids was lowest in sample 5. A peak amount was found in sample 3, approximately the mid portion of the horny layer (Fig. 6).

Discussion

The increased conductance with increasing RH reflects the enhanced water content of the SC as reported by Blank [2] and Middleton [9] in vitro and by us using the in vivo simulation model for SC [11]. However, the conductance values on the lower surface were significantly less than on the upper surface in the range 30–90% RH ($p < 0.01$). Only under extremely humid conditions did this difference disappear. We present here representative data obtained from the samples taken from one individual, but we have confirmed the validity of the data using SC samples prepared from five other individuals.

Decreased conductance could be induced by poor contact between the electrode and the surface of the SC samples. However, such a possibility seems unlikely because we could hardly distinguish macroscopically the lower surface of the SC sheet from the upper. Furthermore, the electrode was applied to the samples with a pressure firm enough to overcome delicate differences in the surface contour if they existed. The isolation procedure of the SC membrane involves scraping off trypsin-digested keratinocytes with a cotton-tipped stick [8]. In

Table 2. The hygroscopicity and desorption rate constant of the upper or lower surface of a stratum corneum sheet mounted with the upper or lower surface upward on the in vivo simulation model

	RH (%)			
	33	69	75	90
Hygroscopicity (μmho)				
Upper surface	394 ± 124*	409 ± 87	465 ± 92	416 ± 130
Lower surface	289 ± 65	403 ± 135	427 ± 77	531 ± 149
Desorption rate constant				
Upper surface	3.4 ± 0.6	2.9 ± 0.4*	2.4 ± 0.3**	1.4 ± 0.3*
Lower surface	3.6 ± 0.5	4.0 ± 1.0	3.1 ± 0.1	1.9 ± 0.3

Values are mean and standard deviation calculated from three experiments. Details of the calculations are described in Reference 14. * $p < 0.05$ and ** $p < 0.001$, significance of difference between upper and lower surface

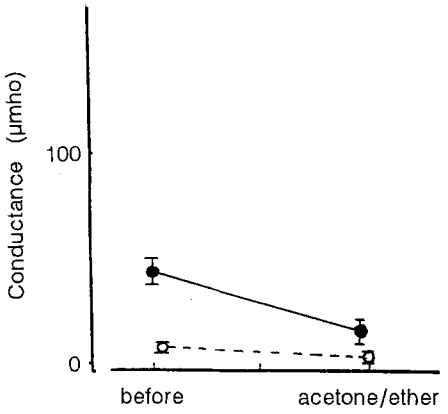

Fig. 4. Change of conductance after lipid extraction with acetone/ether (1:1) for 5 min from the stratum corneum sheet mounted with the upper surface (*closed circles*) or lower surface (*open circles*) upward measured at room temperature (20°C) and 43% RH. A statistically significant decrease in conductance was noted after lipid extraction from the upper surface of the stratum corneum ($p < 0.05$)

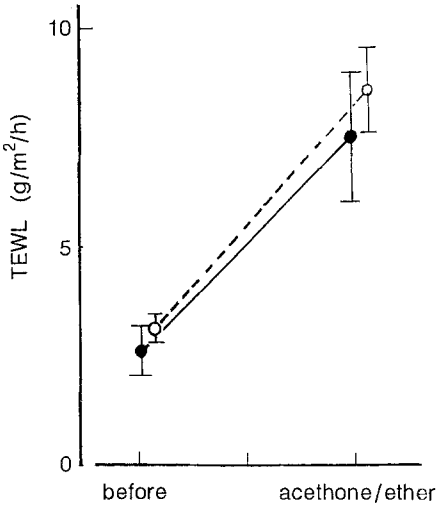

Fig. 5. Change in TEWL after lipid extraction for 5 min with acetone/ether (1:1) from a stratum corneum sheet mounted with the upper surface (*closed circles*) or the lower surface (*open circles*) upward measured at room temperature (20°C) and 43% RH. A statistically significant increase in TEWL ($p < 0.05$) was noted after lipid extraction from both the upper and lower surfaces of the stratum corneum

a preliminary study we found that a similar procedure applied to the upper surface of the SC did not induce any remarkable decrease in conductance or TEWL. Thus, the results suggest that, under ordinary circumstances, the water-holding capacity of the lowest layer of the SC is inferior to that of the uppermost layer. These results were contrary to our expectation.

Functional analysis by the water sorption–desorption test was consistent with these results in that the upper surface was much more capable of binding water than the lower at 69%, 75% and 90% RH, and in this RH range the conductance values showed a greater difference between the upper and lower surfaces than at other RH.

In contrast, TEWL was the same whichever surface was mounted upwards, suggesting that there was no remarkable difference in the water concentration profile in the greater portion of the SC membrane. These data seem to agree well with the conductance data obtained at 97% RH where the upper and lower surfaces of the SC showed similar hydration levels. Under extremely humid conditions, such as occur when the SC sheet faces the water-saturated filter paper, even the lower surface of the SC appears to attain a well-hydrated state.

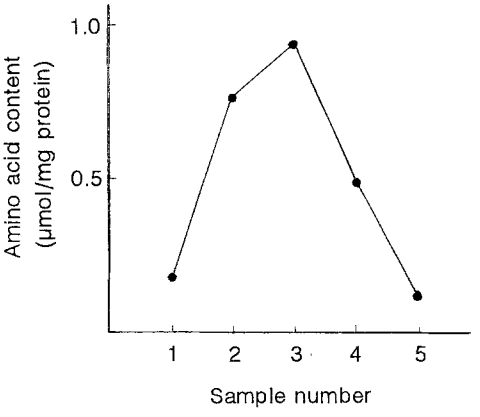

Fig. 6. Amino acid content in serial stratum corneum samples obtained by cellophane tape stripping from the upper surface. Serial strippings were combined into the following samples: (1) strips 1–3; (2) strips 4–6; (3) strips 7–9; (4) strips 10–12; and (5) strip 13

Removal of lipids with acetone/ether induced a significant decrease in the water-holding properties as well as an increase in TEWL, as reported in vivo [6]. This decrease occurred whether the extraction was from the upper or the lower surface, but was particularly apparent in the former case. These data suggest that lipids in the SC play a crucial role in the maintenance of hydration in addition to contributing to barrier properties, especially in the upper portion.

Although unanticipated, our findings indicate that the water-holding capacity of the lower SC is less than the upper surface. Soluble amino acids in the SC are mainly final degradation products derived from keratohyalin granules [9, 13]. They are a major component of water-soluble substances of normal SC, constituting about 40%, and are thought to be related to the water-binding capacity of the SC, because their content in the SC is closely correlated with the hydration state of the SC as well as with the clinical severity of xerosis [5]. Determination of the amount of amino acids at various levels indicated that they were most abundant in the mid portion of the SC and least abundant in the lowermost portion. Although it is impossible to study only the innermost layer of the SC without contamination of the upper layers, the amino acid content of the innermost layer seems to be very low according to the findings of previous studies in which difficulty was found in detecting soluble amino acids in the viable epidermis [4, 12].

The SC is not simply inert, dead tissue: there is ample evidence that active metabolic changes occur in association with the outward passage of corneocytes. Because the hydration of SC prevents the process of filaggrin breakdown to water-binding amino acids [13], the generation of amino acids presumably hardly takes place until the corneocytes move up from the highly hydrated bottom portion of the SC. Similarly, changes in lipid components related to the hydration function as well as to the barrier function take place in different sites within the SC [3], and ongoing hydrolysis of cholesterol sulphate may regulate cell cohesiveness during transit of cells in the SC [19]. In our study the highest concentration of soluble amino acids was noted in the mid portion just below the level at which a single sheet of SC could no longer be obtained by tape-stripping, i.e. just before the stratum dysjunctum. Therefore, from the functional point of view, our data suggest that the SC attains its greatest water-binding capacity in the mid portion, at the upper end of the stratum compactum and that some of this capacity is still retained even at the desquamating upper surface of the SC.

Acknowledgement. We thank A.M. Kligman, M.D., Ph.D., for his critical reading of the manuscript and for many valuable suggestions. This work was supported by Grant-in-Aid for Scientific Research No. 02557044 from the Ministry of Education, Science and Culture, Japan.

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