

Evaluation of out-in skin transparency using a colorimeter and food dye in patients with atopic dermatitis

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Summary

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Conflicts of interest

None declared.

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Background Atopic dermatitis is a disease of skin barrier dysfunction and outside stimuli can cross the skin barrier.

Objectives To examine a new method for evaluating the outside to inside skin transparency with a colorimeter and yellow dyes.

Methods In study 1, a total of 28 volunteer subjects (24 normal and four with atopic dermatitis) participated. After provocation with yellow dye, the skin colour of all the subjects was measured using a colorimeter. The skin transparency index was calculated by the changes of the skin colour to yellow. Other variables of skin function, including transepidermal water loss (TEWL) and stratum corneum hydration, were also measured. In study 2, the skin transparency index was evaluated for a cohort of 38 patients with atopic dermatitis, 27 subjects with dry skin and 29 healthy controls.

Results In study 1, the measurement of skin colour (b^*) using tartrazine showed good results. There was a significant relationship between the skin transparency index with tartrazine and the atopic dermatitis score ($P = 0.014$). No other measurements of skin function, including the TEWL, were correlated. In study 2, the skin transparency index score obtained with tartrazine in the patients with atopic dermatitis was significantly higher than that of the controls and those with dry skin ($P < 0.001$ and $P = 0.022$, respectively). However, the TEWL in patients with atopic dermatitis was not significantly higher than that of patients with dry skin and the TEWL in subjects with dry skin was not higher than that of the controls.

Conclusions This method, which used a colorimeter and food dye, is noninvasive, safe and reliable for the evaluation of out-in skin transparency and can demonstrate the characteristic dysfunction in the skin barrier in patients with atopic dermatitis.

Atopic dermatitis is a common chronic inflammatory skin disease in children.^{1,2} Recent studies suggest that the prevalence of atopic dermatitis is increasing in many countries, as is the prevalence of other allergic diseases.³ Atopic dermatitis commonly begins in infancy or early childhood and patients experience discomfort caused by continuous itching, which induces more scratching and more itching in a vicious circle. The symptoms of itching, scratching and sleeplessness can place a burden on the whole family.

There have been many reports concerning skin dysfunction in atopic dermatitis. Recently a number of reviews have addressed the origin of atopic dermatitis using genetic analysis.^{4,5} The impaired barrier function of the stratum corneum

in atopic dermatitis has been found to result from the decreased production of ceramides⁶ and filaggrin.⁷ Barrier dysfunction induces water loss in the skin and dry skin is a characteristic of atopic dermatitis.

The most common causes of skin itching and exacerbation are sweat and the microparticles of sand and dust that individuals are exposed to in everyday life.^{8,9} In atopic dermatitis, the dysfunction of the stratum corneum seems to be associated with an increased permeability and penetration of environmental irritants and allergens.¹⁰ Stimuli such as sweat and microparticles of sand and dust easily enter into the skin in patients with atopic dermatitis. A reliable evaluation of skin transparency from the outside to the inside of the skin could

indicate an effective strategy for each patient. However, there are few simple, noninvasive methods for objectively evaluating skin transparency.¹¹

The tristimulus photocolourimeter is an instrument for measuring changes in colour by expressing the colour numerically in the L*a*b* colour space, which is the standard of the Commission Internationale de l'Eclairage (CIE) for colour assessment.¹² The CIE L*a*b* colour space can be transformed into other well-known colour systems such as the CIE RGB (red, green, blue) colour space. The system provides an unambiguous description of three coordinates, L*, a* and b*. These indicate the total quantity of lightness or darkness, with the colour ranging from red (positive value) to green (negative value) and from blue (negative value) to yellow (positive value), respectively.¹³

The increased out-in transparency of the skin in atopic dermatitis might induce a continuous transport of outside stimuli across the stratum corneum, resulting in persistent itching and exacerbation of the dermatitis. For a precise evaluation of skin dysfunction a new, simple method of out-in skin transparency measurement using a colorimeter and yellow dye was evaluated.

Patients and methods

Patients

Study 1 was a preliminary trial. Twenty-eight subjects, including 24 normal volunteers who had no skin diseases and no atopic diseases (mean age 19.0 years; male to female ratio 1 : 3) and four patients with atopic dermatitis (mean age 22.6 years, male to female ratio 12 : 12) participated (Table 1).

In study 2, 42 patients with atopic dermatitis (mean age 10.6 years, male to female ratio 19 : 23), 27 patients with dry skin (mean age 18.4 years, male to female ratio 10 : 17) and 26 normal volunteers (mean age 22.3 years, male to female ratio 12 : 14) participated (Table 1). Atopic dermatitis was diagnosed according to the duration, itching and specific skin lesions.¹⁴ The simple atopic dermatitis symptom score (AD score) was used for the evaluation of the severity of atopic dermatitis. The whole body was divided into 25 sections and each section was scored as follows: erosion and heavily

scratched eczema, 2; normal skin, 0; and intermediate, 1. Dry skin was diagnosed as dry skin without a typical atopic dermatitis lesion.

Methods

Measurement of physiological skin functions, stratum corneum hydration and transepidermal water loss

Hydration of the stratum corneum was measured on the flexor aspect of the forearm near the cubital fossa more than 1 h after the patient arrived at the hospital. All tests were performed in a special patients' room with a controlled environment, with the room temperature ranging from 22 to 24 °C and a humidity of 50–60%. Skin temperature remained stable during the examination for all subjects. Stratum corneum hydration was measured using a moisture meter (ASA-M1; Asahi Biomed, Tokyo, Japan),¹⁵ based on capacitance and electrical conductance determined at two different frequencies (160 and 143 kHz) with two concentric surface electrodes. The probe was pressed on to the skin surface for 1–2 s. Each measurement was obtained twice at the same site; the data were rejected when children were crying or visibly sweating.¹⁵ The measurements obtained included the moisture content of the skin surface and the moisture content of the stratum corneum.

All water loss measurements were obtained using the DermaLab modular system with transepidermal water loss (TEWL) probes, which were manufactured by Cortex Technology (Hadsund, Denmark). The TEWL probes contain two sensors that measure the temperature and relative humidity at two fixed points along the axis normal to the skin surface, which allows the device to derive a value electronically corresponding to the evaporative water loss expressed in gram per metre per hour.

The data from the DermaLab modular system were completely computerized and continuously transferred to a personal computer through a serial port using an RS-232C cable and the associated cyber Derm, Inc. software program for the evaporimeters. The application program C1BASIX1 was used to capture the water loss data from the attached evaporimeter at a sampling rate of four inputs per second. These inputs were displayed as graphs in real time on the computer monitor. The extracted value indicated the average rate of evaporative water loss collected over a 20-s interval once steady-state conditions were achieved. The values were directly transferred to an Excel file using a dynamic data exchange (DDE) link. The probe was placed lightly on the skin of the forehead for 1–2 min¹⁶ and the water loss values were electronically recorded using a spreadsheet format based on the Excel software program.

Measurement of out-in skin transparency

The relationship between skin colour and skin transparency was evaluated by using an objective method. A tristimulus

Table 1 Profiles of subjects in studies 1 and 2

	n	M/F	Age (years), mean ± SD
Study 1	28		
Atopic dermatitis	4	1/3	19.0 ± 0.0
Controls	24	12/12	22.6 ± 7.7
Study 2	95		
Atopic dermatitis	42	19/23	10.6 ± 9.3
Dry skin	27	10/17	18.4 ± 14.6
Controls	26	12/14	22.3 ± 8.4

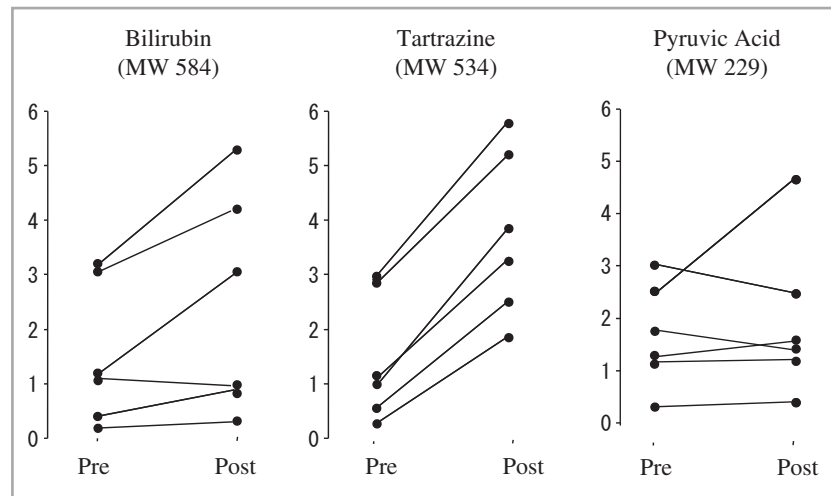


Fig 1. Changes in b^* values (using the standard CIE $L^*a^*b^*$ system) measured with a colorimeter. MW, molecular weight.

photocolorimeter model CR-400 (Konica Minolta, Osaka, Japan)^{13,17} was used to measure objectively the skin colour of the subjects (Fig. 1). This instrument records colours in a three-dimensional space known as the CIE 1976 $L^*a^*b^*$ colour space developed from the CIE XYZ system.^{13,17} The values of L^* , a^* and b^* ranged from 0.0 for black to 100.0 for a diffuse white, from -60 for green to +60 for red and from -60 for blue to +60 for yellow, respectively.¹³ This colour system and the CIE RGB system are interacting.^{13,17} The standard D65 illumination and 2° angle supplementary standard observer were used.

With this instrument the skin surface is illuminated by a pulsed xenon arc lamp. The light that is reflected perpendicular to the surface is collected for a tristimulus colour analysis, using the $L^*a^*b^*$ colour system.

The skin area for the measurement is 11 mm in diameter (95 mm² surface area). The probe is applied to the skin surface of the forearm. To measure the skin colour under the same conditions, the room temperature was kept between 24 and 26 °C.

Study design

In study 1, the time course of the L^* , a^* and b^* values, the selection of dyes and reproducibility of the method were evaluated.

To investigate whether L^* , a^* and b^* values changed with time, they were measured after 5, 10, 15 and 30 min of provocation. The hydrophilic yellow dyes tartrazine ($C_{16}H_9N_4Na_3O_9S_2$, MW 534), bilirubin ($C_{33}H_{36}N_4O_6$, MW 585) and pyruvic acid (2,4,6-trinitrophenol, $C_6H_3N_3O_7$, MW 229) were selected for testing. The dyes were dissolved in saline; all dye solutions were prepared on each trial day. To examine the reliability of the measurement of L^* , a^* and b^* , one doctor measured them in the same subjects twice at an interval of 1 min. To examine the validity of the measurements of L^* , a^* and b^* , two doctors measured them in the same subjects with an interval of 1 min.¹⁸

In study 2, the relationship between the common skin measurements and the skin transparency index, and the skin transparency index with tartrazine and the TEWL were evaluated in controls, subjects with dry skin and patients with atopic dermatitis.

Data analysis

The intraobserver and interobserver reproducibility of measurements (reliability and validity, respectively) were evaluated using the Bland–Altman method and the correlation coefficient. A correlation coefficient of 0.75 was considered as a minimal requirement for the reproducibility of the results. Chronological changes of L^* , a^* and b^* were evaluated by means of Student's *t*-test or a two-way analysis of variance. Correlations between the colorimetric values and the common skin measurements were calculated by a linear regression analysis using Pearson's correlation coefficients. SPSS for Windows release 11.01J (SPSS, Chicago, IL, U.S.A.) was used for the statistical analysis. A parametric analysis of variance was performed to assess any differences in symptom scores between the groups. The data are expressed as the means $\pm$ SD. A *P*-value < 0.05 was considered to indicate a significant difference.

Results

Study 1

Time course of L^* , a^* and b^* values

Fifty microlitres of tartrazine, bilirubin or pyruvic acid solution was applied to a small water-proofed cotton patch (5 × 5 mm) just before attachment to the skin. The L^* , a^* and b^* values changed after provocation with the dyes ($n = 6$, data not shown). For each dye, the values of b^* , which indicates the yellow colour, levelled after 15–30 min of provocation. None of the dyes produced any

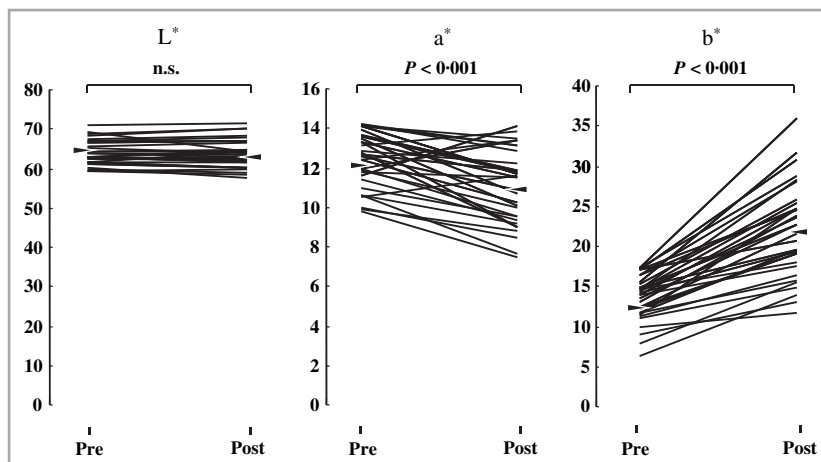


Fig 2. Changes in L*, a*, b* values (using the standard CIE L*a*b* system) after provocation of the skin with tartrazine. Arrowhead indicates mean.

side-effects during a 30-min skin provocation or after the trial. The reliable provocation time was determined to be 30 min.

Selection of dyes

In the measurement of the out-in skin transparency using tartrazine, bilirubin and pyruvic acid (each 10 mg mL⁻¹, 30 min provocation), bilirubin and pyruvic acid demonstrated unreliable data ($P < 0.01$, $n = 6$) (Fig. 1). In all study 1 patients, b* values significantly increased after provocation with tartrazine and the dye showed good penetration into the skin ($n = 28$, $P < 0.001$) (Fig. 2). Therefore, the food dye tartrazine was chosen for this study.

Measurement of out-in skin transparency

The intraobserver correlation coefficient for b* was 0.97, implying small variations within and between observers (Table 2). The repeatability coefficients for intra- and inter-observers for b* were 1.47 and 1.63, respectively. Using the Bland-Altman method, the difference in b* values showed a small variation for both intra- and interobservers (Fig. 3).

Study 2

Relationship between common skin measurements and skin transparency index

An examination of the relationship between the common skin measurements and the skin transparency index was carried out in 42 subjects. The skin transparency index using tartrazine showed a good correlation with the AD score ($P = 0.001$, Table 3, Fig. 4). However, other common skin measurements did not show a relationship with the skin transparency index using tartrazine (Table 3). The moisture content of the skin surface and the stratum corneum significantly correlated ($P < 0.001$, Table 3).

Table 2 Intra- and interobserver variation of skin transparency with tartrazine

Method ^a		SDD	RC	CC
L*	Intraobserver	0.92	2.55	0.94
	Interobserver	0.94	2.59	0.94
a*	Intraobserver	0.87	2.40	0.85
	Interobserver	0.95	2.62	0.77
b*	Intraobserver	0.53	1.47	0.97
	Interobserver	0.59	1.63	0.97

SDD, standard deviation of the difference between the first and second measurements by the same observer (intraobserver) and those by different observers (interobserver); RC, repeatability coefficient (the 95% confidence limit of difference); CC, correlation coefficient.

^aUsing the standard CIE L*a*b* system.

Skin transparency index with tartrazine and transepidermal water loss in controls and patients with dry skin or atopic dermatitis

The differences in these variables were examined between patients with atopic dermatitis, subjects with dry skin and the controls. The skin transparency index using tartrazine in patients with atopic dermatitis was significantly higher than in controls ($P < 0.001$, Fig. 5). In addition, the skin transparency index in patients with atopic dermatitis was significantly higher than in patients with dry skin ($P < 0.022$, Fig. 5). However, the TEWL in the patients with atopic dermatitis was not higher than that of those with dry skin and the TEWL in subjects with dry skin was not higher than that of controls (Fig. 6). There were no significant differences between patients with atopic dermatitis or dry skin and controls in the moisture content of the skin surface and the stratum corneum (data not shown).

Discussion

Recently, several reviews have addressed the origin of atopic dermatitis using genetic analysis^{4,5} and a dysfunction of the

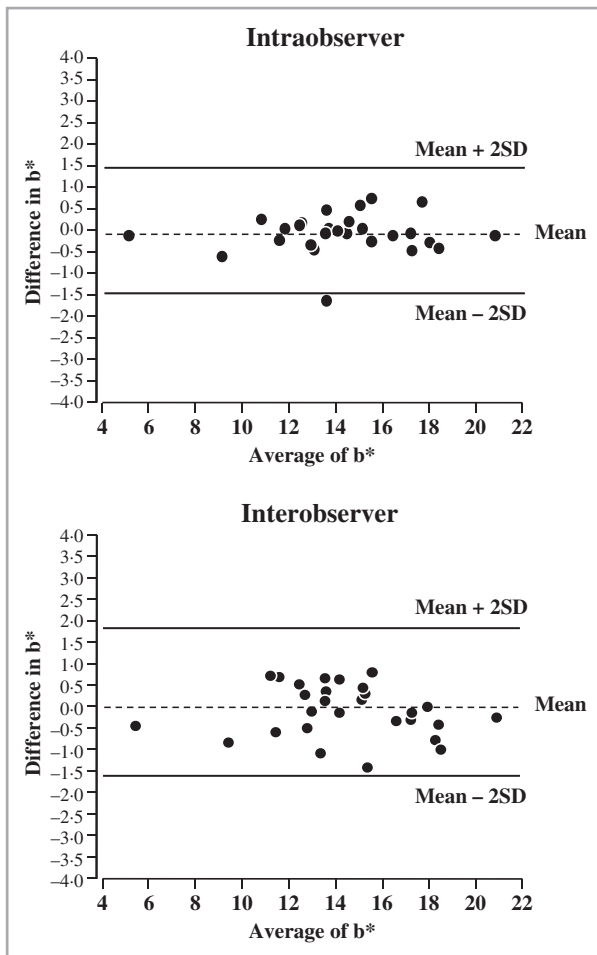


Fig 3. Bland-Altman plots for intra- and interobserver variability of skin transparency with tartrazine.

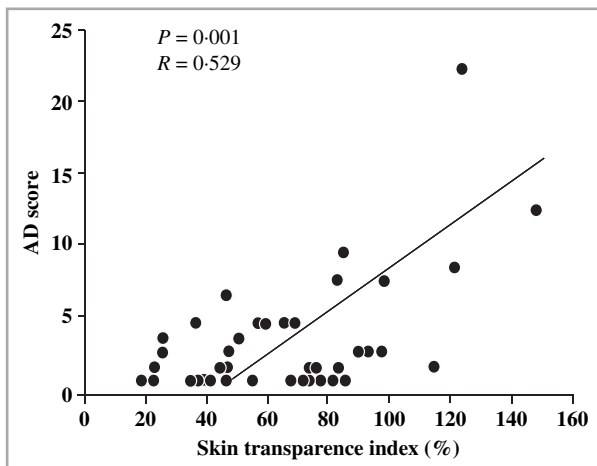


Fig 4. Relationship between the skin transparency with tartrazine and the atopic dermatitis (AD) score in patients with atopic dermatitis.

skin barrier has been suggested to be one of the fundamental mechanisms of atopic dermatitis.^{19,20} Dysfunction of the skin barrier is most often demonstrated by measurement of stratum corneum hydration and TEWL,²¹ which mainly evaluates the

Table 3 Pearson's coefficients between variables in patients with atopic dermatitis (AD)

	STI	TEWL	P	W	AD score
STI		-0.022	-0.125	-0.265	0.529*
TEWL			-0.547**	0.101	0.136
P				-0.541**	-0.190
W					-0.247
AD score					

STI, skin transparency index; TEWL, transepidermal water loss; P, moisture content on the skin surface; W, moisture content in the stratum corneum.
* $P = 0.001$; ** $P < 0.001$.

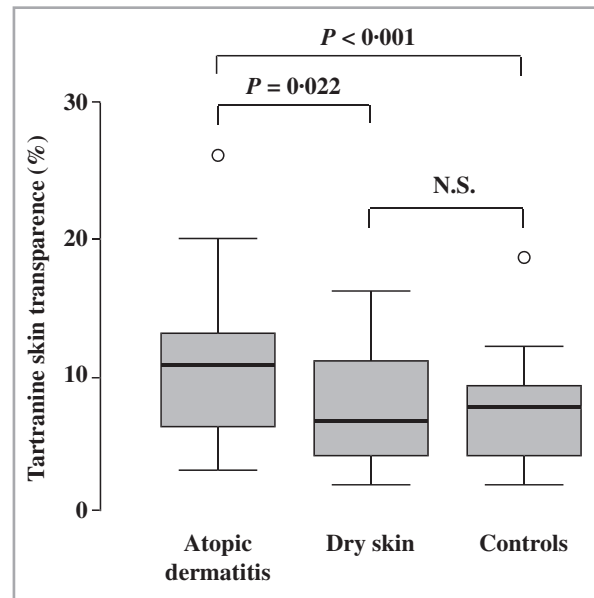


Fig 5. Tartrazine skin transparency in each clinical group. N.S., not significant.

dysfunction of in-out skin transparency. Hata *et al.*¹¹ monitored the noninvasive, objective out-in measurements of skin function. Their method is excellent, but is difficult for routine measurements in children with atopic dermatitis.

Previously, Post *et al.*²² measured the skin reflectance of red, green and blue light in 99 Caucasian infants and reported that the reflectance of each light wave increased with gestational age. The CIE $L^*a^*b^*$ colour space was developed as a standard of the colour space to be used for the specification of colour differences. Watanabe *et al.*¹⁸ found that L^* , an indicator of the darkness or lightness, using a tristimulus photocolormeter strongly correlated with the gestational age.

This colorimeter was used to evaluate a new, unique method for the evaluation of direct out-in skin transparency. The skin colour measurement before and after provocation of the skin with food dye using the CIE $L^*a^*b^*$ colour space colorimeter was assessed and it demonstrated that the b^* value, the indicator of yellow, strongly indicated the out-in

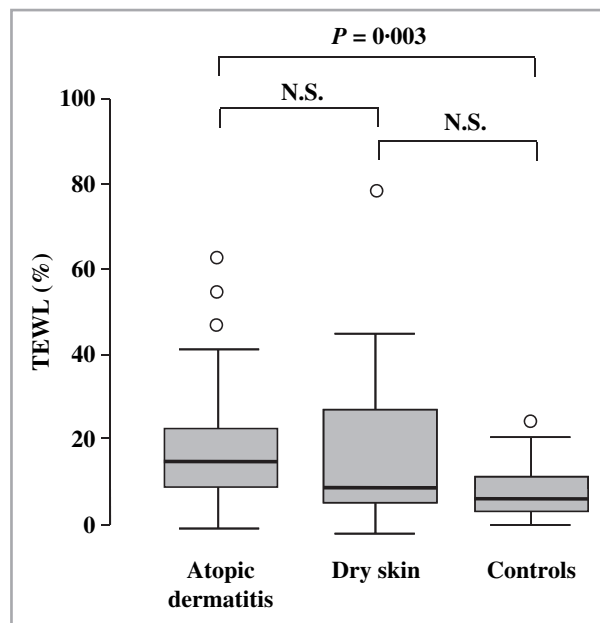


Fig 6. Transepidermal water loss (TEWL) in each clinical group.

skin penetration of the hydrophilic yellow food dye, tartrazine. In addition, the results showed that both intra- and interobserver correlations were satisfactory for b*. This method was simple and reliable enough to measure the skin transparency.

This study demonstrated that the skin transparency index of tartrazine in patients with atopic dermatitis is apparently higher than that in subjects with dry skin and controls and that the skin transparency index in patients with a high AD score is higher than in those with a low AD score. These results suggest that the out-in skin transparency is significantly increased in atopic dermatitis and especially increased in the severe type and that in patients with atopic dermatitis outer stimuli may easily be transported across the skin surface.

Many scientific studies on atopic dermatitis have established guidelines for the effective management of this condition.^{23,24} These guidelines suggest that basic therapy should consist of avoidance of triggering factors and optimal skin care. It is clear that outside irritations and stimuli to the skin contribute to the onset and the aggravation of atopic dermatitis.²⁵ One of the irritants is allergens which induces allergic inflammation in atopic patients.^{26,27} Studies on the effects of house dust mite exposure on eczema have demonstrated specific effects of different mite species.²⁸ Other reported nonspecific irritants are sweat,⁸ sand dust⁹ and staphylococcal enterotoxin.²⁹

Clinically, the symptoms in most young patients with atopic dermatitis are exacerbated after exercise and playing during and/or after school, and taking a shower or bath showed a good result for the treatment of atopic dermatitis.³⁰ One can easily speculate that increased out-in skin transparency could induce chronic skin inflammation and/or prolonged aggravation in patients with atopic dermatitis. In addition, there is the danger theory, i.e. nonspecific irritants stimulate dendritic

cells, resulting in the induction of immunological advance reactions. This supports the mechanism that uric acid in sweat may exacerbate the itch-scratch cycle with skin inflammation in atopic dermatitis.^{31,32} The measurement of out-in skin transparency can be useful for the evaluation of skin physiology in atopic dermatitis and using this method can thus allow the physician to make a more precise plan for the long-term treatment of each individual patient with atopic dermatitis.

Furthermore, the skin transparency index of patients with atopic dermatitis showed a significant difference from that of patients with dry skin, who had the same dry skin but without eczema lesions. TEWL, which mainly indicates the in-out skin transparency, showed no difference between patients with atopic dermatitis and those with dry skin and between subjects with dry skin and controls. The defect of the barrier function in atopic dermatitis has been demonstrated by an increased TEWL measured with an evaporimeter, which evaluates the evaporation rate of water through the skin.³³ Although previous data demonstrate a close correlation between the values of TEWL and the penetration of chemicals through the skin *in vitro*,³⁴ it should be noted that the diffusion of evaporated water molecules through the stratum corneum represents only an in-out barrier function and does not necessarily correlate with the diffusion of proteins and chemicals that often affect the inflammatory processes of atopic dermatitis.¹¹

The current data suggest an apparent difference in skin physiological function between atopic dermatitis and dry skin. The characteristic lesions of atopic dermatitis may be induced by a specific dysfunction in the patients who have skin disorders in in-out and out-in skin transparency. Considering these results, we therefore speculate that there are two types of skin barrier dysfunction: one is the dysfunction of in-out skin transparency, while the other is the dysfunction of in-out and out-in skin transparency. The latter may occur in atopic dermatitis, while the former may occur in dry skin. The dysfunction of in-out and out-in skin transparency, which may be induced by the large spaces that exist among the skin cells, could show more severe, chronic symptoms due to both water loss and nonspecific irritants.

This result is very interesting with regard to the mechanism of onset and the establishment of atopic dermatitis. As clinically normal skin in patients with atopic dermatitis may have minimal inflammation,³⁵ the current observations suggest the possibility of masked skin inflammation which induces a prolonged barrier dysfunction in atopic dermatitis. Although no precise mechanism of the underlying dysfunction in the skin barrier related to out-in skin transparency is indicated, the differential diagnosis of atopic dermatitis from dry skin, which has little dysfunction of out-in skin transparency could be undertaken using this method. This indicates that this method is more useful than TEWL for evaluating the barrier function of the skin.

The dry skin associated with atopic dermatitis, leading to disruption of the skin barrier, has attracted attention as a

nonallergic aetiological factor in atopic dermatitis.⁶ Several studies have demonstrated a lower water-holding capacity in the visually 'uninvolved' skin of children with atopic dermatitis in comparison with children without atopic dermatitis.³⁶ The earliest lesions of infantile atopic dermatitis are erythematous weepy patches on the cheeks, with subsequent extension to the rest of the face and neck. With increasing age, there is a tendency toward drying and thickening of the skin in the involved areas. In infants, a positive relationship has been suggested between stratum corneum hydration and TEWL.³⁷ Furthermore, the current method using a colorimeter is available for estimating dysfunction of the skin barrier in newborns. Evaluating the skin dysfunction in newborns using these measurements is recommended in order to permit effective management in the early stage of life. This report determined the out-in skin transparency only on the forehead and the subjects were 2–39 years old. A new study is planned utilizing the same protocol with younger subjects and using several skin areas.

In conclusion, a useful method for determining the out-in skin transparency was evaluated and it showed a significant increase of out-in skin transparency in patients with atopic dermatitis, which was not found in patients with dry skin or in control subjects. The new method, using a colorimeter and yellow dyes, provides measurements of the barrier function distinct from those related to water evaporation. The development of atopic dermatitis is thought to depend on a complex interplay of genetic factors, environmental exposure to allergens and nonspecific adjuvant factors. However, only a few prospective birth cohort studies have addressed atopic dermatitis occurring in the first year of life.^{38,39} This unique, simple method should be habitually performed for the evaluation of skin dysfunction and the prediction of exacerbation of atopic dermatitis in infants and younger children.

Acknowledgments

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