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A Three-hour Test for Rapid Comparison of Effects of Moisturizers and Active Constituents (Urea)

Measurement of hydration, scaling and skin surface lipidization by noninvasive techniques

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An in vivo skin test for rapid comparison of the efficacy of moisturizers is introduced. Test substances were applied to flexor side forearm skin, and measurements by non-invasive techniques were performed after 3 h. As indicators of epidermal hydration, the electrical conductance and capacitance were measured. To quantify the effects on scale pattern, D-Squame® tapes were scored and the optical transmission measured. Moreover, skin surface lipids resulting from lotion lipids were measured. Lotions and different concentrations of urea could be ranked in a consistent way, viz. untreated skin < vehicle < 3% urea lotion < 10% urea lotion. Urea was concluded to be very potent as a skin humidifier and as a descaling agent, particularly in 10% concentration. Humidity characteristics correlated much better with the concentration of urea than with lipidization of the skin surface due to lotion lipids. A 3-hour test period was sufficient for the evaluation of the effects of moisturizers on both epidermal hydration and scale pattern. With a rapid test method, sources of variation such as diurnal and day-to-day variations, dosage and non-compliance are easily controlled. Further, by using non-invasive methods, the assessment has been rendered objective. ***Key words: moisturizer, urea, hydration, conductance, capacitance, scaling, skin surface lipids, noninvasive, bioengineering.***

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The epidermis is, in most respects, living tissue having active cell functions and a hydration kinetic controlled by the organism. The outermost layers of the epidermis, which ultimately undergo desquamation, adapt passively to environmental conditions.

The outer epidermis is nevertheless very important for the various barrier functions of the skin.

The water barrier of the skin is not a kind of membrane or filter within the epidermis, but a water vapour pressure gradient that transects the skin and a 10 mm layer adjacent of ambient air (1). It is now known that the compartment of the epidermis which is able to adapt to various external humidity conditions is relatively small, and that adjustments take place within 10–15 min, even after immersion in water (2,3,4,5). Following a single application of a moisturizing lotion, with no special additive, the humidity of the epidermis adjusts to the new and improved situation within 10 min, and remains improved for some hours, while elimination of the lotion from the skin surface takes place. This is then followed by a better appearance with fewer scales (6,7).

Thus, based on a modern concept of rapid epidermal hydration kinetics, it should be possible to assess moisturizer effects reliably with a rapid test method. Tagami et al. have already studied the initial absorption – desorption phase and the water-binding capacity by using conductance measurements (2).

The passage and maturation of keratinocytes from the stratum basale to the skin surface takes about 3 weeks. Cohesion between cells is determined by structural elements such as hemidesmosomes and desmosomes. Elias, however, studied the water-lipid intercellular bilayers, which were abnormal in ichthyosis (8). It is still not clearly known how water, moisturizers and urea interact with this bilayer, or what the consequences for cellular cohesion and desquamation might be. However, changes in the intracellular matrix may be induced quite quickly and influence the process of scaling. Thus, it is possible for the treatment effects of moisturizers and urea to be detected after only 3 h.

The purpose of the present study, employing different noninvasive techniques, has been to observe the effects of various moisturizers, including urea lotions, on both the hydration and scaling of the skin, and to develop and evaluate a new and rapid test method. A reduction in scaling is important for both dermatologists and cosmetologists in their choices of treatment.

MATERIALS AND METHODS

Twenty-six healthy volunteers (18 female, 8 male) were studied. Their mean age was 36.3 years (range 21–58 years). One volunteer had suffered from mild psoriasis. Seven had complaints of dry skin and used moisturizers regularly, 11 used moisturizers intermittently, and 8 had no complaints of dryness.

Flexor side forearm skin was studied. Seven different substances (Table I) were tested, and pretest recordings and measurements in an untreated control area were performed. Each forearm was divided into four 25 × 45 mm test areas, separated by 10 mm Micropore® tape to avoid lateral spread of test material. 50 microliters of each test substance was applied by means of a Finnpipette®, mounted with exchangeable tips, having openings approximately chosen dependent on the viscosity of each substance, so as to ensure reproducible dosages, taking into account the viscosity of each material. Tips were washed and dried between applications. Application was made in the centre of each test area. Immediately after application, while still wet, the test material was spread in the test area with a specially made 10 mm wide microroller, intended to produce a uniform film. Emulsion water was allowed to evaporate, and the test region was covered by a single layer of medical gauze as a protection for and from clothing and as a "non-touch" reminder for the volunteers.

The test substances were randomized and coded with respect to both application field and an equal distribution of proximal and distal applications of each substance.

After 3 h (mean 192 min, SD 8 min) systematic measurements were performed.

Table I. *Substances tested*

Code	Substance	Manufacturer
A	ACO fuktlotion®	ACO
B	Nivea lotion®	Beiersdorf
C	L300 ansiktscreme®	Mölnlycke
D	HTH lotion original® (10% urea)	Mölnlycke
E	HTH lotion original® (3% urea)	Mölnlycke
F	HTH lotion original® (urea-free vehicle)	Mölnlycke
G	HTH light®	Mölnlycke

The hydration of the superficial skin layers was measured using the Skicon-100® skin surface hydrometer (IBS Co, Tokyo, Japan), developed by Tagami and co-workers as a prototype (2,9,10). This piece of equipment registers electrical conductance, expressed in reciprocal micro-Ohm. The measured value is displayed digitally, 3 s after starting the measurement. The probe consists of a central 1 mm electrode surrounded by a circular electrode 5 mm in outer diameter. The electrode was applied with a standard load of 30 g. Each recorded value represents the mean value of three measurements.

The hydration of the epidermis, including the lower compartments, was measured using the Corneometer CM-420® (Courage & Khazaka GmbH, Cologne, Germany), (9,10). This apparatus measures the electrical capacitance of the outer skin, expressed in arbitrary units (a.u.). Values are displayed digitally after 3 s. The probe electrode consists of a 16 mm brass grid, covered with an insulating film. The probe was applied under a standard load, and electrical contact with the skin was made via the probe housing. Each recorded value represents the mean value of three measurements.

The scale pattern was studied by means of D-Squame® tape (CuDerm Corp., Dallas, USA). This tape has a diameter of 22 mm. Tapes were applied gently to the test areas, pressed against the skin under a standard load for 5 s, removed carefully at an angle of 45°, using forceps, and mounted on microscope slides (11). Scoring of scaling was performed using a medical viewer, type GR-8 (Hoken Shizai Hanbai Ltd, Tokyo, Japan). The criteria for scoring were as follows: 0, nil scales; 1, slight scaling; 2, moderate scaling and visible skin surface micro relief; 3, severe scaling with a more uniform and heavy distribution of scales. Half-scores were added where samples fell between integral scores. The translucence of the tapes was measured with a Mavolux® digital light measurer (Gossen GmbH, Erlangen, Germany), with values expressed in lux. Unused tapes have a translucence of 84 lux. With scaling, the translucence is lower, and diminishes as scaling becomes heavier. The scoring of scaling and the measurements of translucence represent the average results of two tapes taken from the same test area.

Lipidization of the skin surface, resulting from cream lipids spread over the skin, was measured using the Sebumeter SM 410® (Courage & Khazaka GmbH, Cologne, Germany). This is a photometric device. An opalescent plastic film is pressed against the skin surface for 30 s, and lipids adhere to the film and make it more transparent. This is measured optically, and expressed in arbitrary units which can the be converted into microg/cm² according to manufacturer's calibration table. Values in this study represented the result of one measurement only in each case.

The composition of test substances (Table I) was as follows:

Substance A (ACO fuktlotion®)

2-pyrrolidone-5-carboxylic acid (PCA), glycerin, isopropylmyristate, peanut oil, mineral oil, diethanolamine(DEA)-cetyl phosphate, polyethyleneglycol(PEG)-2-monostearate, stearic acid, diethanolamine, alcohol, methylparaben, propylparaben, butylhydroxytoluene (BHT), fragrance, water. The lipid content is 9%, and the pH 5.5.

Substance B (Nivea lotion®)

A declaration of contents is not available. The lipid content is 16.5%, and the pH is 6.4.

Substance C (L300 ansiktscreme®)

Urea 4%, sodium chloride 4%, mineral oil, PEG-5 stearyl stearate, cetearyl alcohol, stearic acid, tromethamine, sorbic acid, fragrance, water. The lipid content is 21.5%, and the pH is 6.0.

Table II. *Results (mean value and SD of non-invasive measurements and scoring taken 3 hours after application of test substances A, B, C, D, E, F and G to forearm skin.*
P = pretest control value, K = untreated skin control value

		CONTROL		SUBSTANCE						
		P	K	A	B	C	D	E	F	G
Efficacy										
Epidermal hydration										
conductance,	mean	12.32	12.65	36.04	15.56	18.55	58.64	43.97	17.20	44.80
1/μΩ	SD	7.05	8.03	21.93	7.35	7.43	26.78	20.22	7.39	20.41
capacitance,	mean	83.93	85.24	100.46	91.63	98.62	102.47	101.69	93.52	102.26
a.u.	SD	7.66	7.98	8.27	8.81	8.22	15.71	15.29	6.95	16.85
Scaling (D-Squame®)										
score	mean	–	2.64	1.37	2.04	1.04	0.15	1.29	1.79	1.33
	SD	–	0.30	0.46	0.37	0.45	0.37	0.35	0.35	0.35
transmission, lux	mean	–	58.81	70.23	61.96	72.39	78.12	73.42	66.15	72.46
	SD	–	5.06	6.73	5.49	4.13	1.90	4.41	5.93	4.94
Lipidization										
Skin surface lipids										
lipids,	mean	0.35	0.35	31.85	37.60	39.58	10.58	11.77	4.07	18.65
μg/cm²	SD	0.51	0.70	12.15	15.02	15.02	5.49	6.59	3.83	9.22

Table III. *Numbers of volunteers with reduced, same or increased values according to paired comparison (n = 26)*
Untreated control (K) and skin treated with vehicle (F); skin treated with vehicle (F) and 3% urea lotion (E); 3% urea lotion (E) and 10% urea lotion (D)

	Untreated skin/vehicle			Vehicle/3% urea lotion			3% urea lotion/10% urea lotion		
	reduced	Same	Increased	Reduced	Same	Increased	Reduced	Same	Increased
Epidermal hydration									
conductance	3	0	23	0	0	26	4	1	21
capacitance	4	0	22	2	0	24	14	3	9
Scaling (D-Squame^R)									
score	25	1	0	22	3	1	26	0	0
transmission	2	1	23	2	0	24	1	0	25

Substance D (HTH-lotion original 10% carbamide^R)

Urea 10%, lactic acid 5%, betaine, propylene glycol, cetearyl alcohol, ethylhexyl ethylhexonate, tromethamine, glyceryl stearate SE, DEA-cetyl phosphate, dimethicone, fragrance, water. The lipid content is 6%, and the pH 3.5%.

Substance E (HTH-lotion original 3% carbamide^R)

Urea 3%, lactic acid 1.5%, betaine and other constituents as for substance D.

Substance F (HTH-lotion original^R cream base)

Urea 0%, lactic acid 0%, betaine 0%, other constituents as for substance D.

Substance G (HTH-lotion light^R)

Urea 3%, lactic acid 1.5%, betaine, propylene glycol, mineral oil, PEG-5 stearyl stearate, ethylhexyl ethylhexonate, steareth-21, cetearyl alcohol, glyceryl stearate self-emulsifying, tromethamine, fragrance, water. The lipid content is 14%, and the pH is 3.5.

The study took place during the winter (March). The temperature in the laboratory was 20–22° C and the mean relative humidity was 43%. The volunteers performed their normal tasks during the treatment period. Prior to measurements they had to wait in the laboratory for 10–15 min with the test region uncovered. They were not allowed to use skin care products during the test, and they should not have used emollients for 3 days prior to the experiment. Otherwise they were given no special restrictions.

Statistical analysis was performed with the paired t-test. $p<0.05$ was considered significant. Substances D (10% urea), E (3% urea) and F (vehicle), and the control area (K) and pretest recordings (P) were subjected to analysis. Substances or products with other vehicles were also evaluated and ranked.

RESULTS

Results of the measurements of epidermal hydration, the assessment of scaling and the measurement of lipidization of the skin surface due to lotion lipids are presented in Table II. The numbers of volunteers (total n=26) with reduced, unchanged or increased values after treatments with lotions F (vehicle), E (3% urea) and D (10% urea) are presented in Table III, with the untreated control as a reference.

The statistical analysis of hydration (conductance and capacitance) and of scaling (scoring and the measurement of translucence of D-Squame^R tapes) showed improved hydration and reduced scaling consistently when vehicle and untreated skin were compared ($p<0.01$), when E (3% urea) and vehicle were compared, and when D (10% urea) and E (3% urea) were compared. The only exception noted was that the capacitance measurements for E and D were effectively the same (n.s.). Thus, a clear dose – response relationship was established, with increasing effect from F (vehicle) versus untreated skin, through E (3% urea) versus F, to D (10% urea) versus E.

Figs. 1–5 and Table IV summarize the results and ranking for the different lotions.

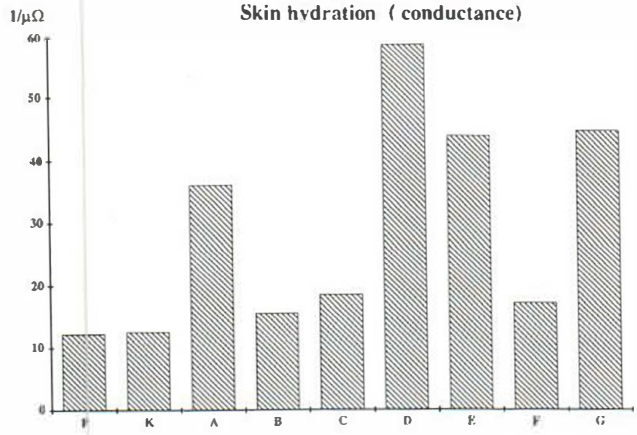

Fig. 1. Hydration of superficial skin layers (mean values) 3 h after treatment with lotions A, B, C, D, E, F and G. Electrical conductance was measured with the Skicon-100^R.

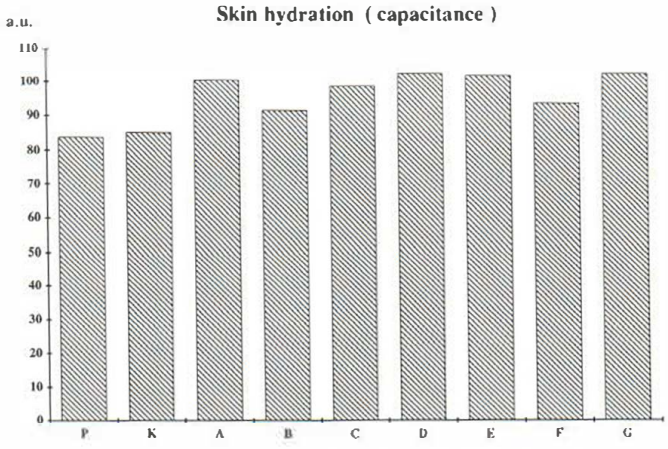

Fig. 2. Hydration of epidermis, including lower compartments (mean values) 3 h after treatment with lotions A, B, C, D, E, F and G. Electrical capacitance was measured with the Corneometer CM-420^R.

Table IV. *Rank order of substances with respect to efficacy (improvement of hydration and reduction of scaling) and with respect to lipidization of the skin surface due to vehicle lipids.*
1 = high efficacy/lipidization. 8 = low efficacy/lipidization

	Order of ranking							
	1	2	3	4	5	6	7	8
Efficacy								
Epidermal hydration								
conductance	D	G	E	A	C	F	B	K
capacitance	D	G	E	A	C	F	B	K
Scaling (D-Squame®)								
score	D	C	E	G	A	F	B	K
transmission	D	E	G	C	A	F	B	K
Lipidization								
Skin surface lipids								
lipids	C	B	A	G	E	D	F	K

Lotions D (10% urea), F (vehicle) and B (Nivea®) and K (representing untreated skin) were ranked consistently. D was highly efficient, and B not very efficient. Lotions containing 3% urea, i.e. C, E and G, grouped into ranks inbetween D and F, with a tendency for A (ACO fuktlotion®) to be less efficient.

Measurement of skin surface lipids closely reflected the lipid content of the lotions, with the exception that G ranked lower than A. There was generally no clear relationship between lipid content and efficacy, in contrast to that regarding urea content.

DISCUSSION

The study demonstrated that a 3-hour rapid test method is useful for ranking the efficacy of moisturizing lotions. Methods based on an electrical principle for the determination of epidermal hydration, and tape method assessments of scaling, showed a high degree of consistency. Thus, the known close relationship between epidermal hydration and scaling and desquamation was confirmed. Urea is a very potent humidifier, particularly in 10% concentration.

In contrast, the study did not confirm any close relationship between skin lipidization, from lotion lipids, and scaling, which might be anticipated from the studies by Elias on the intercellular matrix of the epidermis (8). However, a 3-hour study cannot exclude that lotion lipids may influence the process of desquamation when used for a longer period, and different lipids may exert different effects, depending on their ability to penetrate into the epidermis and amalgamate with the intercellular matrix. Nevertheless, lotion lipids did not appear to be potent in the control of scaling, as demonstrated by the results obtained with lotion B.

As mentioned initially, the hydration of the outer epidermis adjusts rapidly and passively to ambient conditions. A rapid test method has the advantage that diurnal and day-to-day variations of skin hydration kinetics are much easier to control and to keep constant. Furthermore, dosage and laboratory conditions can be carefully standardized and non-compliance avoided. Finally, with the designed test method described here, a number of substances can be compared under con-

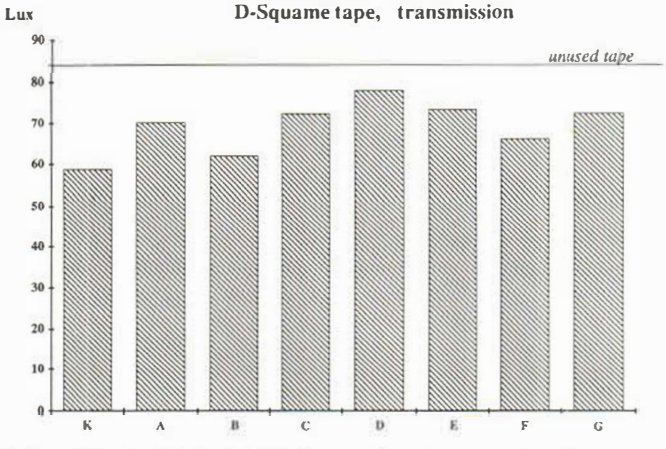

Fig. 3. Scaling according to measurements of translucence with D-Squame® tapes, 3 h after treatment with lotions A, B, C, D, E, F and G. Low translucence indicates heavy scaling, high translucence indicates slight scaling, unused tape = 84 lux.

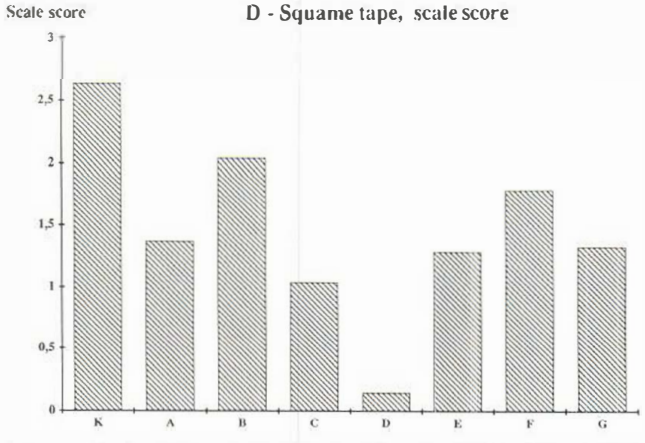

Fig. 4. Scaling according to scoring of D-Squame® tapes, 3 h after treatment with lotions A, B, C, D, E, F and G. 1, slight scaling; 2, moderate scaling; 3, heavy scaling.

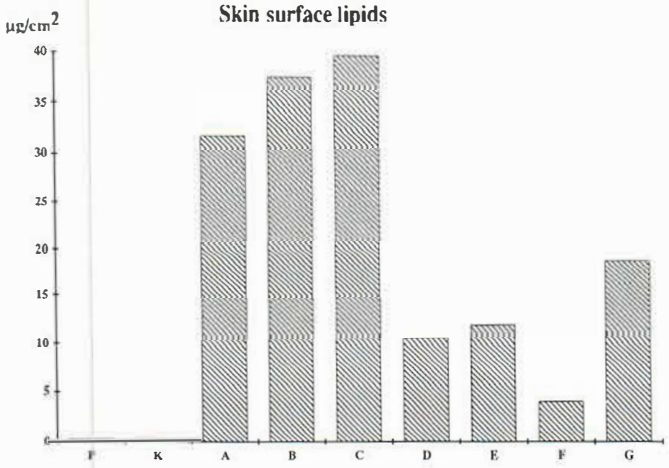

Fig. 5. Skin surface lipids 3 h after applications of lotions A, B, C, D, E, F and G.

cealed and randomized conditions and, with objective measurements of the results using non-invasive techniques.

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