Effects of Repeated Application of a Moisturizer

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Epidermal hydration following repeated application of an oil in water emulsion was studied on the forearm skin of 16 healthy females by non-invasive methods. The lotion was applied twice daily for 7 days, and values were followed 7 days after cessation of treatment. The opposite forearm served as an untreated control. Electrical conductance and capacitance showed similar results, i.e. increased values ($p < 0.001$) after 2 days of application, reaching a plateau during further applications. Two days after cessation, values were still increased ($p < 0.001$), and the conductance was also increased 7 days after cessation of treatment. The water evaporation and the cutaneous blood flow did not change, i.e. indicating no mild irritant effect. Skin surface lipids did not change, i.e. indicating that no significant amounts of emulsion oil remained on the skin at the time of recording. Probably components of the oil phase of the emulsion are absorbed into the epidermis, which is associated with improved hydration as a later event. ***Key words: Electrical conductance; Electrical capacitance; Transepidermal water loss; Cutaneous blood flow; Skin surface lipids.***

(Accepted April 10, 1989.)

Acta Derm Venereol (Stockh) 1989; 69: 457–459.

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Emulsions are widely used in dermatology as moisturizing agents and as vehicles for active substances. However, knowledge about their physiological effects on skin is still limited.

In a recent study with a 6-hour observation period, we showed that single application of an oil in water emulsion affected the skin in a diphasic way (1). Immediately after application there was an *evaporation phase*, which lasted less than 15 min and was dominated by evaporation of emulsion water. This was followed by a *lipidization phase,* which lasted at least 6 h and was dominated by emulsion oil and its gradual absorption, associated with an improvement of epidermal hydration parameters, i.e. electrical conductance and capacitance.

In the present study, the same oil in water emulsion was applied for 7 days, and parameters of skin physiology followed during application as well as 7 days after treatment by non-invasive methods.

MATERIAL AND METHODS

Subjects

Sixteen healthy women, 23–58 years of age, participated. They were instructed not to use skin care products for at least 2 days before testing and for 7 days afterwards. Normal washing with a bar soap was permitted. Physical and psychological stress was avoided 30 min before measurements.

Test area

After randomization, the test substance was applied to the flexor aspect of one forearm, with the opposite forearm serving as control. A test area measuring 6×18 cm was designated.

Test substance

Each volunteer was supplied with 14 syringes containing 0.4 ml oil-in-water emulsion (Decubal® lotion) to be used twice daily for a 7-day period, one syringe each morning after washing, another one at night before bed. The emulsion was spread in an even layer over the area using one finger. The calculated thickness of the lotion layer was 37 μm. In the oil phase the test emulsion contained cetanol, anhydrous lanolin (Westbrook Lanolin Company, UK), isopropyl myristate, glycerol and Span 60® (sorbitan stearate). The water phase consisted of Tween 60® (polysorbate 60), methyl-*p*-hydroxybenzoate, propyl-*p*-hydroxybenzoate, and aqua purificata. The final lotion contained 80.4% of water.

Measurements

Measurements were performed in the morning between 9 and 11 a.m., i.e. about 12 h after the last application of test substance. A pre-test value was obtained on the first day, and further measurements were performed after 2, 7 (at cessation of applications), 9, and 14 days (follow-up). Thus, the subjects were followed until one week after the last application, each measure followed at exactly the same site of the forearm after at random division of the test area into smaller compartments dedicated to each parameter. Control measurements were done strictly symmetrically.

Recordings were performed in a laboratory room with constant temperature (20–23°C) and humidity. Attempts were made to avoid convection of air. Doors and windows were kept closed. Subjects were asked to refrain from walking or talking. The study was carried out between March and April 1987.

Measurement equipment

Electrical conductance was measured by the Skicon-100® 3.5 MHz (I.B.S. Ltd, Tokyo) skin surface hydrometer (2, 3),

Table I. *Repeated application of a moisturizer twice daily (days 0–7) to forearm skin (n=16); untreated follow-up on days 9 and 14; results of assessment by bioengineering methods*

E=emulsion treated side, C=contralateral control. Differences between E and C and standard error of the mean are given.

Day ...		0	2	7	9	14
Electrical conductance (1/μohm)	E mean	12.1	27.7	36.0	25.8	19.0
	C mean	11.3	10.2	11.6	10.4	14.7
	E-C mean	0.7	17.5**	24.4***	15.4***	4.3*
	E-C SEM	0.97	3.62	2.62	2.31	1.65
Electrical capacitance (a.u.)	E mean	63.6	75.8	81.0	74.5	67.9
	C mean	62.8	64.4	63.5	64.3	65.6
	E-C mean	0.8	11.4***	17.5***	10.2***	2.3
	E-C SEM	1.50	1.69	1.95	1.60	1.39
Water evaporation ($g/m^2/h$)	E mean	3.4		4.8		3.6
	C mean	3.1		4.4		3.5
	E-C mean	0.3		0.4		0.2
	E-C SEM	0.30		0.60		0.42
Skin surface lipids ($\mu g/cm^2$)	E mean	0	0.3	0.2	0.2	0
	C mean	0	0	0	0.1	0
	E-C mean	0	0.3	0.2	0.1	0
	E-C SEM	0	0.17	0.10	0.09	0
Cutaneous blood flow (a.u.)	E mean	19.6		23.8		21.9
	C mean	20.8		24.1		22.5
	E-C mean	−1.1		−0.3		−0.6
	E-C SEM	1.57		1.89		2.14

*$p<0.02$, **$p<0.01$, ***$p<0.001$.

electrical capacitance by the Corneometer CM 410® (Schwarzhaupt GmbH, Cologne) (3), water evaporation from the skin surface by the Servo Med EPI® (Servo Med AB, Stockholm) evaporimeter (4, 5), skin surface lipids including emulsion oil by the Sebumeter® (Schwarzhaupt GmbH, Cologne) (6), and cutaneous blood flow by the Periflux® (Perimed AB, Stockholm) laser Doppler flowmeter (7).

All measurements were performed at two different sites, and the result expressed as average values.

Water evaporation and laser Doppler flow, which served as supplementary information, were only measured on days 0, 7 and 14.

Statistical methods

Statistical analyses were carried out by the Student's *t*-test for paired observations by A. Mørup Jensen, M.Sc. The emulsion-treated side was compared with the control side of the same subject at the same time. *P*-values less than 0.05 were considered significant if not specified.

RESULTS

Results of the study are shown in Table I, which includes mean differences between treated sides and their respective controls as well as the standard error of mean differences.

Significant increases were found in electrical conductance on days 2 ($p<0.01$), 7 ($p<0.001$), 9 ($p<0.001$), and 14 ($p<0.02$). The study also showed significant increases in electrical capacitance on days 2 ($p<0.001$), 7 ($p<0.001$), and 9 ($p<0.001$), while there was no difference on day 14. There was no difference in evaporation, cutaneous blood flow or skin surface lipids at any time.

DISCUSSION

Both parameters of epidermal hydration, i.e. electrical conductance and electrical capacitance, showed consistently that the epidermal hydration was improved during treatment with the test lotion. The Skicon-100® measures very superficially, while the CM 410 Corneometer measures the epidermal hydration down to about 0.1 mm (3). Thus, the improvement of hydration concerns both the stratum corneum and outer parts of the stratum granulosum.

This is in accordance with our initial study on single-application of the same lotion indicating gradual epidermal absorption of emulsion lipids during a

6-h period after application (1). Emulsion water evaporates within 15 min after an application (1, 8). In this study we found no relation between skin surface (emulsion) lipids and the electrical measures indicating no direct and significant influence of the test lotion itself on the electrical measures. Measurements of the skin surface lipids at the time of recording showed no significant increase. The improvement in parameters of hydration found in the present study was therefore probably a result of a biological effect of emulsion oil on the outer epidermal layers. The structure of the outer epidermis, with liposomes forming an intercellular lipid-rich cement between keratinocytes, renders such a hypothesis likely (9).

Evaporimeter and laser Doppler flowmeter measurements indicated that the changes in the hydration parameters were not due to the mild irritation from the lotion with a defect in the water barrier of the skin or inflammatory vasodilation (10, 11).

Although the lotion studied was rather thinly applied, with a water content of about 80%, only two daily applications resulted in a significant improvement of the hydration characteristics, reaching a plateau after 2 days of treatment. It is interesting that this alteration was rather protracted, lasting at least 7 days after cessation of a one-week treatment.

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