Effects of Single Application of a Moisturizer: Evaporation of Emulsion Water, Skin Surface Temperature, Electrical Conductance, Electrical Capacitance, and Skin Surface (Emulsion) Lipids

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Effects of single application of an oil in water emulsion were studied on the forearm skin of 12 healthy volunteers. Five different non-invasive methods were used. Values were followed for 360 min after application of the emulsion, with the contralateral forearm as untreated control. The evaporation of emulsion water from the skin surface immediately rose to high values, but within 15 min returned to the original level. A parallel initial increase in conductance was observed; however, this was followed by a slightly increased level throughout the 360 min study. Electrical capacitance was also slightly increased throughout the study. Skin surface lipids, dominated by emulsion lipids, were increased, with high values for at least 120 min, followed by a gradual decline toward normal. Single application of emulsion is characterized by an initial *evaporation phase*, with evaporation of emulsion water, which lasts less than 15 min, followed by a *lipidization phase*, which lasts at least 360 min, dominated by the oil-constituent of the emulsion undergoing epidermal absorption. During the lipidization phase, epidermal hydration parameters are slightly but consistently improved.

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Moisturizing emulsions are frequently used in dermatology in the treatment of a variety of diseases such as atopic dermatitis. Emulsions are also popular as vehicles for active substances in local treatments. However, knowledge about the physiological effects of moisturizers and cream bases is still limited.

In the present study, we combined a number of new and non-invasive methods to follow physiological changes in the skin resulting from single application of an oil in water emulsion.

MATERIAL AND METHODS

Twelve healthy females, aged 45–66 years, were studied. They were asked not to use any skin care product for 2 days before testing. They could continue normal personal washing with a bar soap. Physical and mental stress was avoided 30 min before measurements.

The flexor aspects of both forearms was used. A test area measuring 6×18 cm was indicated on each side, and the area subdivided into 7 equal areas. Measurements were performed, after randomization, either in the distal or in the proximal direction 3–5, 15, 30, 60, 120, 240 and 360 min. after application of the test substance. Prevalues were recorded from the distal, middle and proximal thirds of the forearm immediately before application.

Test lotion (0.4 ml) was spread over the 6×18 cm test area. This gives a calculated lotion thickness of 37 μm. The lotion was applied to only one arm, after randomization, with the contralateral arm serving as control. Measurements were made symmetrically during the experiment. Decubal®, an oil in water lotion, was used as test substance. The oil phase consists of cetanol, anhydrous lanolin P-95 (Westbrook Lanolin Company, UK), isopropyl myristate and Span 60® (sorbitan stearate). The water phase consists of glycerol, Tween 60® (polysorbate 60), methyl-p-hydroxybenzoate, propyl-p-hydroxybenzoate, and aqua purificata. The water content of the test emulsion was 80.4%.

Evaporation from the skin surface (transepidermal water loss and evaporation of emulsion water) was measured by the Servo Med Evaporimeter EP1® (Servo Med AB, Stockholm) (1, 2), electrical conductance by the Skicon100® 3.5 MHz skin surface hydrometer (IBS Ltd., Tokyo) (3, 4), electrical capacitance by the Corneometer CM 420® (Schwarzhaupt GmbH, Cologne) (4), skin surface lipids by the Sebumeter® (Schwarzhaupt GmbH, Cologne) (5), and skin surface temperature by the Comark 200l® (Comark Electronics Ltd., Rustington, UK) contact thermometer. Determination of skin surface lipids was based on optical transmission of a skin surface imprint obtained with a frosted plastic foil.

Recordings were performed in a laboratory room with temperature 20–23°C and constant humidity. Attempts were made to avoid convection of air. Participants were asked to refrain from walking or talking. The study was performed during December 1986.

Analysis was carried out by the Student's *t*-test for paired observations by A. Mørup Jensen MSc (Dumex A/S, Copenhagen). *p*-values less than 0.05 were considered significant.

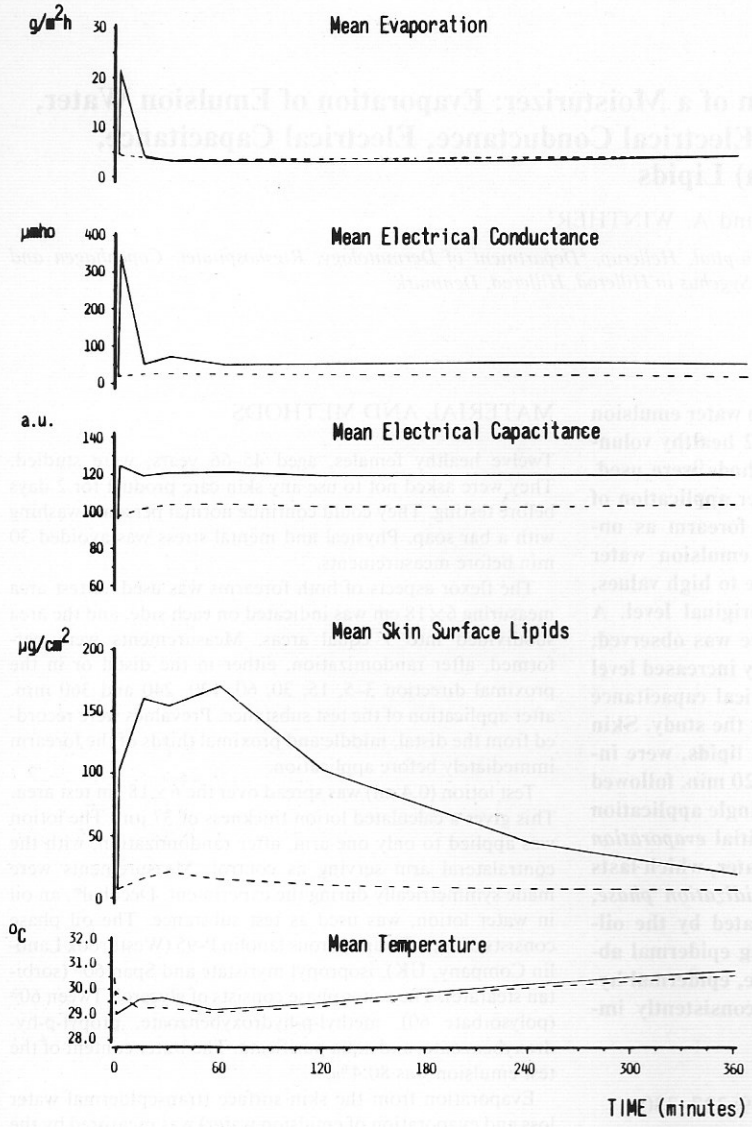

Fig. 1. Summary of results. Mean values of evaporation, electrical conductance, electrical capacitance, skin surface lipids and skin temperature in 12 subjects. Successive measurements during a 360 min. period after application of an emulsion. Contralateral control indicated by ---. Distribution of observations, see Table I.

RESULTS

Results are given in Table I. The evaporation was significantly increased immediately after application of the test substance. After 15 min, the evaporation was already equal to the control side. The electrical conductance showed a great increase, analogous to the evaporation curve. After 15 min, conductance values dropped to a level which was, however, still significantly increased in comparison with contralateral control throughout the 360 min. experiment. The electrical capacitance was significantly increased throughout the whole experiment with values holding at a plateau. Measurement of skin surface lipids showed a broad peak between 0 and 120 min followed by a gradual decline toward zero during the examination period. Skin surface temperature did not change significantly. Values gradually increased during the day.

Fig. 1 gives a summary (mean values, ranges not indicated) of parameters assessed and their relation. Median temperature tended to decrease immediately after application of substance, followed by a slight and constant increase. The initial decrease probably reflected evaporation of emulsion water.

Statistical analysis of pre-test values showed no right-left differences and no systematic proximal-distal difference of the area of forearm skin studied.

Table I. *Successive measurements (median and range) during a 360 min. period following application of a moisturizer*

Time 0 = prevalue, E = emulsion, C = control of contralateral forearm

			Time (min)							
			0	1	15	30	60	120	240	360
Water evaporation (g/m^2 h)	E	Median	3.5	16.5	4.0	3.0	4.0	3.0	3.5	3.5
		Range	2–7	6–48	2–8	1–7	0–5	2–6	2–7	2–7
	C	Median	4.0	4.0	3.5	2.5	3.0	3.0	3.0	3.5
		Range	0–9	2–8	2–7	1–9	1–5	0–6	1–6	2–7
Electrical conductance (1/μohm)	E	Median	13.5	354.5	41.0	52.0	51.0	53.0	54.5	58.0
		Range	5–33	145–644	30–83	22–310	30–84	30–101	32–126	18–131
	C	Median	19.5	19.0	23.0	25.5	26.0	26.5	21.0	20.0
		Range	7–51	10–27	9–51	9–60	15–47	8–60	9–73	11–71
Electrical capacitance (a. u.)	E	Median	98.5	126.0	121.0	121.0	123.5	120.0	119.5	119.0
		Range	84–104	114–139	106–132	111–130	108–128	109–130	105–129	104–126
	C	Median	96.5	99.0	105.0	102.5	104.5	100.5	99.5	101.5
		Range	83–112	84–107	83–111	85–115	92–114	93–115	88–115	88–113
Skin surface lipids ($\mu g/cm^2$)	E	Median	0.0	98.5	183.0	162.5	208.0	109.5	37.0	7.0
		Range	0–3	11–181	7–279	59–271	74–360	9–236	2–88	0–53
	C	Median	0.0	0.0	5.5	13.5	7.0	3.0	1.0	2.0
		Range	0–4	0–13	0–38	0–73	0–49	0–27	0–15	0–5
Skin surface temperature (°C)	E	Median	30.6	29.1	29.8	30.0	29.7	29.7	30.8	31.2
		Range	28.8–31.7	26.9–30.4	27.0–31.5	27.5–31.4	27.4–30.6	28.3–31.0	28.4–32.0	28.3–32.7
	C	Median	30.5	30.1	29.2	29.5	29.1	29.3	30.6	31.2
		Range	28.6–32.2	27.6–31.5	27.5–32.3	26.8–31.4	27.4–31.2	27.5–31.1	28.5–31.9	28.3–32.0

DISCUSSION

This study indicates that physiological changes following single application of an oil in water emulsion can be diveded into two distinct phases, i.e. an initial *evaporation phase*, which lasts less than 15 min, dominated by evaporation of emulsion water, and a *lipidization phase*, lasting at least 360 min and dominated by the oil phase of the emulsion, associated with minor improvements of hydration parameters. The gradual and slow clearing of skin surface lipids or oil during the lipidization phase cannot be explained by evaporation of lipids, and it is not likely to be due to invisible desquamation since lipids bind small particles. Thus, during the lipidization phase, emulsion lipids probably penetrate into the outer epidermis, and this is associated with increments in electrical conductance and capacitance. The alteration of these hydration parameters cannot be explained by external supply from the emulsion water since diffusional equilibrium between the skin surface and the ambient air takes place within a few minutes (6). The intercellular lipid-rich compartment of the epidermis is of significance for the barrier of the skin (7). It is likely that emulsion lipids penetrate the outer epidermis and mix up with this compartment with consequences for epidermal hydration and scaling. Thus, in a cream base or lotion the water phase seems only of importance as a vehicle, and the oil phase seems to exert the therapeutic effects.

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Acitretin Induces an Increased Adherence of *S. Aureus* to Epithelial Cells

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Recently, synthetic retinoids have been implicated as causing a rise in the incidence of staphylococcal infections in patients orally treated with these compounds for various disorders of keratinization. Since the adherence of bacteria to epithelia is an important early event in the development of bacterial infections, in the present study we investigated the in vitro effects of acitretin on the adherence of *Staphylococcus aureus* to epithelial cells of the anterior nares of 15 healthy human subjects. It was found that pre-incubation of nasal epithelial cells with acitretin causes a statistically significant ($p < 0.001$) increase in the adherence of *S. aureus* to these cells, as compared to the controls. The growth of *S. aureus* cultures in the presence of acitretin exerted no effect on the staphylococcal adherence. These results suggest that the oral acitretin-induced increase in *S. aureus* colonization and in the incidence of cutaneous staphylococcal infections may be related to the enhancement of staphylococcal adherence to epithelia caused by this compound.

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Staphylococcus aureus is a bacterial pathogen of major concern in dermatology since it is the most common cause of cutaneous infections (1). Recently, isotretinoin and etretinate have been implicated as causing an increased colonization of anterior nares by *S. aureus* and a rise in the incidence of staphylococcal infections in patients orally treated with these compounds for various disorders of keratinization (2, 3, 4).

Adherence of bacteria is an important early event in the bacterial colonization of epithelial surfaces and in the pathogenesis of the subsequent infections (5, 6, 7). Furthermore, selective adherence seems to be responsible for species and tissue bacterial tropisms (1).

Since acitretin, the main metabolite of etretinate, shares very similar side-effects with the parent drug (8) in the present study we investigated the in vitro effects of acitretin on the adherence of *S. aureus* to epithelial cells of the anterior nares of healthy human subjects.

MATERIAL AND METHODS

Epithelial suspensions

Epithelial cells were obtained from the anterior nares of 15 healthy volunteers by gently scraping with a wooden tongue depressor and suspended in phosphate buffered saline (PBS: 0.07 M phosphate; 0.15 M NaCl, pH 7.4). More than 95% of the cells were viable, as indicated by their ability to exclude 0.4% trypan blue. For the bacterial adherence assays we used epithelial cells pre-incubated with 450 ng/ml acitretin (in 1% DMSO) for 30 min. In preliminary experiments we found no significant differences between the effects of acitretin in concentrations of 450, 800 and 1 300 ng/ml. Epithelial cells pre-incubated in 1% DMSO without acitretin served as controls.

Bacterial suspensions

A clinical strain of *S. aureus* was grown overnight (18 h) at 37°C in nutrient broth containing 450 ng/ml acitretin. Cultures without acitretin were used as controls. All cultures were then centrifuged, washed and suspended in PBS at a final concentration of 10^8 bacteria/ml.

Bacterial adherence assay

The ability of the test organism to bind to epithelial cells was tested as previously described (9). The epithelial cells were