

**Biophysical Properties of
Dry Atopic and Normal Skin
with Special Reference to Effects of
Skin Care Products**

BY
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ABSTRACT

During recent years several highly developed non-invasive methods for evaluation of skin physiology and pathology have been introduced. Against this background, the present studies were undertaken with the primary aim of assessing the effects of various skin care products on some properties of the skin. Skin topography was measured by profilometry on skin replicas, friction with a newly developed friction instrument, capacitance with a Corneometer, and barrier function both with an Evaporimeter to assess transepidermal water loss (TEWL) and by application of an irritant followed by measurement of the resulting irritative reaction.

Initially some of the techniques were used to further characterize the differences between dry atopic skin and normal skin. Dry skin exhibits increased values of roughness parameters and a reduced number of topographical peaks. TEWL is increased, indicating impaired barrier function. The friction and capacitance are lower and correlate significantly to each other, whereas TEWL does not appear to relate to either of these parameters.

The use of a scrub cream removes the outermost part of the stratum corneum, resulting in a smoother skin. Application of moisturizers modifies the frictional response of the skin. The friction instrument gave results comparable to those of panellists trained in sensory evaluation. The study suggests that measurement of skin friction can be used to predict the degree of liking of moisturizers.

Furthermore, moisturizers increase the skin hydration. They provide water directly to the skin from their water phase. Skin hydration also increases with increased degree of occlusion, as measured as a decrease in TEWL. Moisturizers may also alter the diffusional resistance of the stratum corneum and reduce the skin susceptibility to the surfactant sodium lauryl sulphate (SLS).

Lipids in moisturizers may influence already developed SLS-induced irritation. A significantly lower degree of irritation was found in areas treated with canola oil and its sterol-enriched fraction than in an area treated with water.

These findings emphasize that skin care products do not only form an inert, epicutaneous layer, but that they may penetrate and influence the structure and function of the skin.

Key words: humans, dermatology, moisturizers, lipids, bioengineering methods, sodium lauryl sulphate, TEWL, LDV, stratum corneum, nonsaponifiable lipids.

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PAPERS DISCUSSED

The thesis is based on the following papers, which are referred to in the text by their Roman numerals:

- I. Linde YW, Bengtsson A, Lodén M. "Dry" skin in atopic dermatitis. II. A surface profilometric study. *Acta Derm Venereol (Stockh)* 1989; 69: 315-319.
- II. Lodén M, Olsson H, Axéll T, Linde YW. Friction, capacitance and transepidermal water loss (TEWL) in dry atopic and normal skin. *Br J Dermatol* 1992; 126: 137-141
- III. Lodén M, Bengtsson A. Mechanical removal of the superficial portion of the stratum corneum by a scrub cream: Methods for the objective assessment of the effects. *J Soc Cosmet Chem* 1990; 41: 111-121.
- IV. Lodén M, Olsson H, Skare L, Axéll T. Instrumental and sensory evaluation of the frictional response of the skin following a single application of five moisturizing creams. *J Soc Cosmet Chem* 1992; 43: 13-20.
- V. Lodén M, Lindberg M. The influence of a single application of different moisturizers on the skin capacitance. *Acta Derm Venereol (Stockh)* 1991; 71: 79-82.
- VI. Lodén M. The increase in skin hydration after application of emollients with different amounts of lipids. *Acta Derm Venereol (Stockh)* 1992; 72: 327-330.
- VII. Lodén M. Urea containing moisturizers influence the barrier properties of human skin. Submitted for publication.
- VIII. Lodén M, Andersson A-C. The effect of topically applied lipids on surfactant-induced irritation. Accepted for publication 1995 in *Br J Dermatol*.

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ABBREVIATIONS

EFA	Essential fatty acid
GLA	Gamma linolenic acid
NMF	Natural moisturizing factor
PCA	Pyrrolidone carboxylic acid
SEM	Scanning electron microscopy
SLS	Sodium lauryl sulphate
TEWL	Transepidermal water loss
UV	Ultraviolet

INTRODUCTION

The skin

The skin - the interface between man and the environment - is the largest organ in the body. It weighs an average of 4 kg and covers an area of 2 m². Death from loss of skin, as in a burn, the problems of inflammatory skin disorders, e.g. psoriasis and eczema, and the misery of unpleasant pimples, all remind us of its many important functions, which range from the vital to the cosmetic.

The functions of skin

The skin protects the body against physical injury and prevents loss of body water and other substances, and thus assists in keeping the internal environment constant. Likewise, it prevents foreign chemical substances and microbes from entering the body. Light, too, is screened by the outermost layers of the skin. It has also been demonstrated that the skin is an important immune organ, acting as an immunological barrier. Besides being a barrier, it functions as a link between the internal and external environments. Through the various sensory organs in the skin, the afferent system informs the central nervous system about the external environment. The sensory nerve endings respond to touch, pressure, changes in temperature and other stimuli. The skin also has a thermoregulatory role, since the cutaneous blood flow and the secretion of sweat can be controlled. The skin must also be regarded as having endocrine functions, since it is a site of production of vitamin D₃ and metabolism of a number of steroids.

The skin is also an important organ of communication (Thody 1986). Touching the skin is essential in numerous aspects of parental and sexual behaviour. The appearance and texture of our skin are also prominent factors in the skin's communicatory role. Embarrassment may be revealed by flushing, while pallor occurs during anxiety and anger. It is possible that the use of cosmetics enhances our social communication and that perfumes serve as substitutes for odours which our bodies do not produce.

Skin morphology

The skin consists essentially of two layers: an outer stratified squamous epithelium, the epidermis; and an inner layer, the dermis, containing superficial blood vessels, cutaneous nerves, sweat glands, sebaceous glands and hair follicles, all held together by connective tissue. The dermis provides tensile strength to the skin, and this property is primarily attributable to the effects of collagen and elastin, which are present in the dermis in a fibrous interwoven meshwork.

The epidermis is 50-150 µm thick. Its principal cells are the keratinocytes, whose main purpose is to produce the tough and almost impermeable outer layer, the stratum corneum. Thus, the epidermis is being continually replaced and is thus well adapted to its requirements for repairing damage from wear and tear. During the process of epidermal differentiation, synthesis of keratin and stratum corneum lipids occurs, and these proteins and lipids provide the structural and chemical integrity of the stratum corneum (Smack 1994, Downing 1992). A functioning stratum corneum is essential for human survival in a dry environment. The stratum corneum covers the whole body surface and is able to stay soft and flexible in the usual ambient conditions, allowing free body movement without producing any cracking or fissuring on the skin surface. The epidermis is also the frontier of the immune system by virtue of the antigen-presenting function of the Langerhans' cells and the various immunomodulatory cytokines secreted by these cells and by the keratinocytes. In addition, the epidermis contains melanocytes which produce melanin for protection against UV radiation.

Surface features

The most visible features of normal skin are wrinkles. These are situated in particular sites, for example the expression lines of the face, and folds around the joints. They vary in depth from 0.1 mm to several millimeters depending on age and environmental factors. A less pronounced system of furrows can be observed on any region of the body. The furrows intersect each other in complex ways and create a variety of geometrical patterns (Piérard-Franchimont 1987, Tring 1974, Lavker 1980). Closer examination of the intermediate plateaus shows the presence of secondary, finer furrow systems (Piérard-

Franchimont 1987, Tring 1974, Lavker 1980, Wagner 1979). Higher magnification reveals the arrangement of the corneocytes and their morphological features (Marks 1971). The geometrical organization of the skin surface varies from one region to another and may be related to local mechanical requirements (Wagner 1979, Lavker 1980). Differences in the surface pattern relate to the size of the polyangular units and the depth of furrows (Wagner 1979). For instance, the skin over the elbows, knees and knuckles is characterized by large polyangular units, deep furrows and complex folds which allow the necessary mobility of the skin (Wagner 1979). Another prominent feature in these areas is the presence of discrete epidermal papillae, which give the surface a cobblestone appearance (Wagner 1979).

For centuries dermatologists have depended on their eyes and fingers to assess visible and tactile changes of the skin. However, with the use of macrophotography (Wagner 1979) or scanning electron microscopy (SEM) of skin replicas (Ryan 1983) or skin surface biopsy specimens (Piérard-Franchimont 1987, Marks 1971) a greater understanding of the skin surface features has been obtained. For instance, the ageing process induces alterations in the skin surface pattern, which are more pronounced in exposed areas than in protected skin (Lavker 1980). The observations on cutaneous microtopography can also be graded visually to obtain an index of actinic skin damage (Holman 1984). Both in skin diseases (Linde 1989a, Piérard-Franchimont 1987, Kuokkanen 1972, Holman 1984, Serup 1986) and after application of irritants (Agner 1987), characteristic changes of the skin topography are observed.

The frictional characteristics of the skin provide us with information regarding its texture, its suppleness and smoothness, and its dryness or oiliness. Friction is also a frequent contributor to skin abrasions and blisters (Sulzberger 1966).

Biology of the stratum corneum

Structurally the stratum corneum can be likened to a brick wall, with the approximately 20 stacked layers of corneocytes representing the "bricks" and the lipid intercellular matrix the "mortar" (Elias 1981b). During the life cycle of the keratinocytes, these cells differentiate: they gradually flatten out, lose their nuclei and other organelles, and eventually completely cornify to become corneocytes which are filled with keratin and amorphous matrix. In the transition to stratum corneum, intracellular lipids are extruded by

exocytosis from the keratinocytes. The turnover time for normal epidermis, including the stratum corneum, has been calculated to be about 52 to 75 days (Halprin 1976). This is an indirect measure of the proliferative activity in the epidermis. In skin with higher activity, the projected size of the flattened corneocytes becomes smaller (Grove 1983). This size has been found to be important for the permeability barrier function (see below) (Rouquier 1988, Denda 1994, Potts 1991).

Water content and natural moisturizing factor, NMF

Water is known to be an important constituent of the stratum corneum in maintaining its softness and flexibility (Blank 1952, 1953). If a piece of cornified epithelium is dried out, it becomes hard and brittle, and cannot be softened by immersion in any lipid substance, such as petrolatum, lanolin or vegetable oil (Blank 1952). Water is also required for the enzymatic hydrolysis of lipids and other skin constituents.

Water in the stratum corneum is associated with the hydrophilic parts of the intercellular lipids and with the keratin fibres in the corneocytes (Elias 1981b, Takenouchi 1986). The fibrous elements in the corneocytes have hydrophilic properties and also contain a water soluble fraction which enhances their water holding capacity (Middleton 1978, Laden 1967a, Blank 1953). In the hydrated stratum corneum three types of water with different molecular mobilities can be found. At a water content below 10% the primary water is tightly bound, presumably to the polar sites of the proteins (Anderson 1973, Hansen 1972, Takenouchi 1986). When the degree of hydration exceeds 10%, the secondary water is hydrogen-bonded around the protein-bound water, and above 40-50% the water resembles the bulk liquid (Hansen 1972, Takenouchi 1986, Anderson 1973). It is the secondary water that contributes to the plasticity of the stratum corneum (Takenouchi 1986, Blank 1953).

The water soluble fraction of the low molecular humectants is termed "natural moisturizing factor" (NMF) and accounts for 15 to 20% of the total weight of the corneum (Jacobi 1959, Laden 1967a). The composition of NMF in normal skin is shown in Table 1. If NMF is extracted from the skin, then the ability of the stratum corneum to bind water decreases (Laden 1967ab, Blank 1953). Pyrrolidone carboxylic acid (PCA) occurs primarily in the stratum corneum in the form of its sodium salt at levels reaching about 3 to 4% (Laden 1967b). NMF not only seems to be important for the water holding capacity of skin, but it also appears to increase the stratum corneum elasticity (Imokawa 1991b, Middleton 1974, Jokura 1992).

Thus, if NMF is removed, water alone cannot restore elasticity (*Jokura 1992*).

Table 1. Composition of natural moisturizing factor.

	(%)
Amino acids	40.0
Pyrrolidone carboxylic acid	12.0
Lactate	12.0
Urea	7.0
Na, Ca, K, Mg, phosphate, chloride	18.5
NH ₃ , uric acid, glucosamine, creatinine	1.5
Rest unidentified	

Source: Jacobi 1959.

Intercellular lipids

The lipid composition of the epidermis changes dramatically during epidermal differentiation (*Yardley 1981, Wertz 1989*). There is a marked decrease in phospholipids and an increase in fatty acids and ceramides (*Yardley 1981, Wertz 1989*). In the final stages of this differentiation, keratinocytes discharge lipid-containing granules - lamellar bodies - into the extracellular spaces in the upper granular layer, where they form intercellular membrane bilayers (Fig. 1) (*Wertz 1989, Downing 1987, Elias 1977, 1981b*). This lamellar material greatly expands the intercellular compartment and it constitutes about 5-10% of the total weight of human stratum corneum (*Elias 1981a, Lampe 1983*). The composition of these lipids is

unusual. Unlike the lipids in all other biological membranes, those in the stratum corneum do not contain phospholipids, but are mainly composed of ceramides, sterols and fatty acids (Table 2).

The lipid composition varies in different regions of the skin (*Lampe 1983*). Furthermore, the distribution of ceramides appears to be sex- and age-dependent (*Denda 1993, Rawlings 1993b, Imokawa 1991a*). The season also seems to influence the lipid composition of the normal stratum corneum (*Rawlings 1993b, 1994*). For instance, the proportions of ceramides, cholesterol and fatty acids are significantly lower in the winter and spring months of the year than in the summer (*Rawlings 1993b, 1994*). Especially the ceramide esterified to linoleic acid has been found to be decreased in the winter (*Rawlings 1993b*).

The content and organization of these lipids have broad implications for the permeability barrier function, water retention and desquamation (*Scheuplein 1971, Downing 1987, Yardley 1981, Elias 1977, 1981ab*). These factors are probably related to the capacity of the lipids to form multiple lipid bilayers. It has been suggested that the observed seasonal variation in the lipid composition may indicate that the stratum corneum is functionally inferior in the winter and that this could explain the known susceptibility to skin xerosis and faulty desquamation at this time of the year (*Rawlings 1994*). As early as in 1968, Middleton showed that powdering the stratum corneum destroyed the lipid membranes and made the skin more susceptible to drying out effects (*Middleton 1968*).

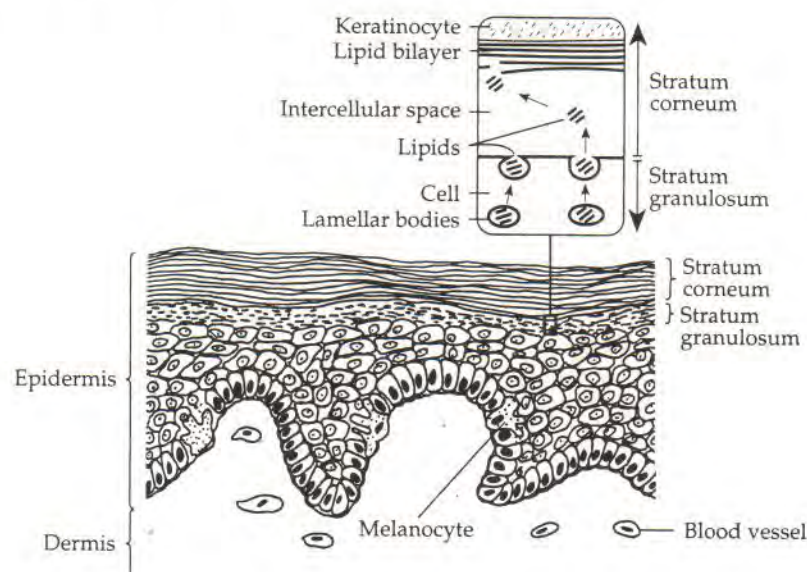


Figure 1. Structure of the epidermis and a schematic presentation of the formation of the intercellular lipid bilayer.

Table 2. Composition of human stratum corneum lipids.

Lipid	Data from Lampe 1983 (facial skin)	Data from Wertz 1989
Ceramides	19.9	39.1
Fatty acids	19.7	9.1
Triglycerides	13.5	0.0
Free sterols	17.3	26.9
Cholesteryl esters		10.0
Cholesteryl sulphate		1.9
Sterol/wax esters	6.2	
Squalene, n-alkanes	9.7	
Others	6.7	11.1

Permeability barrier of the skin

The structural arrangement of the lipid molecules in the transverse plane is not clearly elucidated, but recent studies suggest that the different lipids may segregate in the membrane and form separate fluid and solid phases within the stratum corneum (White 1988, Knutson 1987). Forslind (1994) has proposed that the bulk of the lipids are in crystalline/gel domains bordered by lipids in a fluid crystalline state and called this arrangement a "domain mosaic model". He proposes that this model is an effective water barrier which allows a controlled loss of water to keep the corneocytes moistened (Forslind 1994).

One of the most important functions of skin is to prevent the loss of body water to the environment. Water reaches the surface of the skin via the sweat ducts and by passive diffusion across the epidermis. The integrity of the stratum corneum determines the diffusional rate of transepidermal water loss (TEWL). TEWL shows interindividual variations (Blichmann 1987) and varies from one area of the body to another (Rougier 1988, van Sam 1994).

Another important barrier function of the skin is to prevent the entry of environmental substances. Most of the substances that come in contact with the skin are efficiently excluded by the stratum corneum. However, any substance applied to the skin may be absorbed. Whether pharmacological or toxicological levels are reached within the skin or in the body depends on the nature of the substance, the integrity of the stratum corneum and the dose applied. The molecular weight and lipophilicity of the applied substance are important factors in determining its penetration (Potts 1992, Scheuplein 1971). Hence, the ability of peptides to penetrate normal skin is more than a thousand times lower than that of formaldehyde (Lodén 1986, 1988).

The total lipid content in the stratum corneum (Elias 1981b), the degree of hydration of this skin layer (Blank 1984, Tiemssen 1989), the size of the corneocytes (Rougier 1988, Potts 1991) and the thickness of the stratum corneum (Scheuplein 1971) are factors that have been shown to be responsible for differences in skin permeability. Among these, the intercellular lipids appear to be the most crucial for the barrier function (Elias 1981b, Potts 1992, Abrams 1993). There are several evidences for this. For instance, the quantities of lipids in a particular skin site are more critical for barrier function than the thickness of the stratum corneum (Elias 1981a). Furthermore, removal of lipids by exposure to organic solvents induces profound alterations in barrier function (Abrams 1993, Grubauer 1987, 1989ab). In addition, the recovery of barrier function which follows lipid removal by solvents or detergent, also parallels the replenishment of stratum corneum lipids (Menon 1985, Grubauer 1987, 1989ab).

That the degree of hydration of the stratum corneum has a profound effect on the skin permeability has been demonstrated by several authors (Tiemssen 1989, Blank 1984, Cooper 1976, Ryatt 1988). It has been suggested that increased permeability may be due to creation of interfacial defects in the lipid bilayer caused by an increased lipid phase separation, since no evidence of an overall increase in the intercellular lipid disorder has been observed (Mak 1991). According to Potts (1991), not only the structure of the bilayer membrane but also the unique morphology of the stratum corneum itself may be important for the permeability. He points out that the highly convoluted and tortuous lipid pathway implies a greater distance for penetration than the actual thickness of the stratum corneum. With a smaller corneocyte area, this tortuosity decreases, which may explain the greater

permeability (Rougier 1988, Denda 1992) and increased susceptibility to chemical irritation observed in skin with smaller corneocytes (Al-Jaberi 1984). With increasing proliferative activity in the epidermis, the size of the corneocytes decreases (Grove 1983, Denda 1992).

The concept of dry skin

Dry and chapped skin is a very common problem both in healthy individuals and in patients with skin diseases, e.g. atopic dermatitis and hand eczema. There are several features that give an impression of dry skin (Lévêque 1987, de Rigal 1993, Linde 1989a, Jemec 1992). The visible and tactile characteristics mentioned below are judged by the dermatologist, while the sensory experiences are also taken into account by the affected person:

- visible features - redness, a lack-lustre surface, dry white patches, flaky appearance, cracks and even fissures;
- tactile features - rough and uneven; and
- sensory characteristics - feels dry, uncomfortable, painful, itchy, stings and tingles.

Roughness and scaling are visible features in areas of clinically dry skin in patients with atopic dermatitis (Linde 1989a). Closer examination of these areas by SEM shows that the surface morphology is changed from a regular pattern to a coarser one with broad, irregularly running furrows and loss of minor furrows (Linde 1989a). Likewise, in xerosis, increasing derangement of minor furrows and later also of major furrows can be observed (Piérard-Franchimont 1987). A more coarse and irregular skin surface pattern with larger squares is also found in recessive X-linked ichthyosis (Kuokkanen 1972). The size of the corneocytes may also be changed, and in the dry skin type known as winter xerosis the projected area decreases with increasing severity of the xerosis (Lévêque 1987).

In studies of winter xerotic skin, the water content of the stratum corneum has been found to correlate inversely with clinical scores of dryness (de Rigal 1993, Lévêque 1987). Elderly patients with xerosis also show a decreased water content in the stratum corneum (Long 1992, Horri 1989). Furthermore, the dry-looking skin of patients with atopic dermatitis and psoriasis is less hydrated and less capable of binding water than normal skin (Werner 1986, Berardesca 1990, Thune 1989, Tagami 1982, Takenouchi 1986, Serup 1987). In vitro studies have also confirmed that pathological stratum corneum from atopic and

psoriatic patients is less capable of binding water than normal stratum corneum (Werner 1982, Takenouchi 1986).

The amount of tightly bound water, which does not seem to have any plasticising effect, is almost the same in different types of pathological skin, whereas the amount of secondary water (see above), accounting for the elasticity, is much smaller in stratum corneum from psoriatic patients and from elderly persons with xerosis than in normal stratum corneum (Takenouchi 1986). For instance, in normal stratum corneum from glabrous skin the content is 38.2 mg/100 mg dry tissue, as compared with 31.7 mg in senile xerosis and 27.2 mg in psoriatic scales per 100 mg dry tissue (Takenouchi 1986). Thus, the occurrence of dry skin may be related to a deficiency of water in the stratum corneum.

However, it has not been conclusively shown that the water content of the stratum corneum is reduced in all dry skin conditions. For instance, measurements indicate that the pruritic and dry-looking skin in patients with chronic renal failure (Stähle-Bäckdahl 1988) and the clinically dry-appearing old skin (Thune 1989) seem to have a normal water content. There is also a discrepancy between the subjective self-assessment and the clinical assessment of the presence of dry skin (Jemec 1992, Linde 1989a). Some authors consider dry skin to belong to a group of disorders with a rough skin surface (Rurangirwa 1987). For instance, the skin in acne patients may be considered dry on account of the rough surface, despite a high sebum output (Rurangirwa 1987, Lévêque 1987, Piérard 1987). Thus, the impression of dryness might not be related to the presence or absence of skin surface lipids (Rurangirwa 1987, Lévêque 1987, Piérard 1987).

Although water is known to play an important role in maintaining skin suppleness and plasticity (Blank 1952), other components may also affect its physical properties. For example, topically applied lipids influence the visible and tactile properties of the skin (Nicholls 1978, Garber 1976, Nacht 1981) and α -hydroxy acids may increase the skin elasticity (Alderson 1984, Takahashi 1985, Hall 1986, Hagan 1993). Furthermore, it has been suggested that glycerine prevents crystallization of stratum corneum lipids and thereby preserves their normal structure when the skin is underhydrated (Froebe 1990).

The causes of clinically dry skin and changes in NMF and lipid composition

Individual and environmental factors interact in a complex manner to produce dry skin. The following factors are important as causes of clinically dry skin:

- environmental factors - low humidity, low temperature;
- behavioural factors- exposure to solvents, cutting fluids, surfactants, acids, alkalis etc; and
- genetic factors - inherited disorders relating to the structure and function of the epidermis, e.g. ichthyosis, atopic dermatitis.

Clinically dry skin may also be secondary to a pathological condition, e.g. diabetes or renal failure.

A deficiency of NMF is linked to dry skin conditions. In ichthyosis vulgaris (Sybert 1985) and psoriasis (Marstein 1973) there is a virtual absence of NMF. The amino acid compositions of stratum corneum samples from old people are altered in xerotic skin (Jacobson 1990, Horii 1989). There is a decrease in the amount of water-soluble amino acids in relation to the severity of xerosis, a finding which has been suggested to reflect decreased profilaggrin production (Horii 1989). A reduced content of amino acids has also been observed in experimentally induced scaly skin (Denda 1992). Furthermore, the stratum corneum in patients with severe ichthyosis vulgaris with a low surface hydration state, has a lower amino acid content than normal stratum corneum (Horii 1989). The content of urea both in the normal and affected stratum corneum of patients with atopic dermatitis is also substantially reduced (Wellner 1992). In addition, a significant relationship has been found between the moisture binding ability and the PCA content of samples of stratum corneum (Laden 1967b).

Besides changes in NMF, the distribution of the intercellular lipids is also altered in several dry skin conditions and in disorders of keratinisation. An abnormal lipid composition has been observed in surfactant-irritated skin (Fulmer 1986), in experimentally induced scaly skin (Denda 1992), in normal winter dry skin (Saint-Léger 1989), in dry atopic skin (Melnik 1988, 1990, Linde 1989b, Imokawa 1991a, Hollmann 1991, Yamamoto 1991), in psoriatic plaques (Motta 1994), in hereditary ichthyosis (Paige 1994) and in essential fatty acid (EFA)-deficient states (Prottey 1976). In particular the content and distribution of the ceramides are changed (Paige 1994, Denda 1992, 1993, Imokawa 1991a, Fulmer 1986, Motta 1994, Melnik 1988, 1990). In addition to the changes in lipid composition, the normal bilayer structure is perturbed in skin dryness (Rawlings 1993c) and in skin exposed to organic solvents (Man 1993, Feingold 1990).

Barrier function and repair

Dry, scaly skin is usually associated with impaired barrier function. Increased TEWL is observed in

surfactant-irritated skin (Grubauer 1987, Denda 1994, Agner 1989, 1990, Seidenari 1994, Lee 1994), in tape strip-induced scaly skin (Denda 1992), in dry atopic skin (Werner 1985, Berardesca 1990, Thune 1989, Finlay 1980), in psoriatic plaques (Motta 1994, Serup 1987) and in EFA-deficient states (Elias 1980, Prottey 1976). An increased susceptibility to chemical irritation has also been observed in patients with atopic and exogenous dermatitis (Al-Jaberi 1984). However, although the skin hydration was found to be reduced in winter xerotic skin, TEWL remained in the normal range (Lévêque 1987).

A disturbance of the epidermal barrier function induces a rapid response of the keratinocytes to restore cutaneous homeostasis. The mRNA coding for pro-inflammatory cytokines, adhesion molecules and growth factors is up-regulated (Nickoloff 1994). Likewise there is an increase in DNA synthesis, leading to epidermal hyperplasia, and in lipid synthesis (Proksch 1993, Grubauer 1987, 1989ab, Menon 1985, Feingold 1990). Recent studies point to TEWL as a possible signal that regulates the lipid synthesis in mice (Grubauer 1989a). For instance in EFA-deficient mice the lipid synthetic activity in the skin is increased (Feingold 1986). Disruption of the barrier function with acetone or detergents will also induce a burst of epidermal lipid synthesis in mice (Holleran 1991, Grubauer 1987, 1989ab, Menon 1985). The synthetic activity includes unsaponifiable lipids (Grubauer 1987, Menon 1985, Feingold 1990), fatty acids (Grubauer 1987) and sphingolipids (Holleran 1991). Sterols and fatty acids are synthesized immediately after barrier disruption, whereas the increase in sphingolipid synthesis is somewhat delayed (Holleran 1991). Over time, the content of lipids in the stratum corneum is restored to the normal level in parallel with the return of barrier function (Grubauer 1987, 1989ab, Menon 1985, Feingold 1990, Holleran 1991). That TEWL may serve as a signal for recovery of barrier structure and function has also been shown in occlusion experiments in mice. Covering of the skin with an occlusive membrane after disruption of the stratum corneum barrier down-regulates the increase in lipogenesis in EFA-deficient mice (Feingold 1986) and in acetone-disrupted mouse skin (Grubauer 1989a).

Bioengineering methods for evaluation of skin properties

During recent years a number of highly developed non-invasive instruments for studies of the physiology and pathology of the skin have been introduced. These

instruments can provide information not otherwise obtainable, e.g. in the analysis of subclinical irritation (Lee 1994, Seidenari 1994, Nilsson 1982) and in other forms of disease (Werner 1985). Kligman (1991) has emphasized the need for quantitative methods that will reveal functional disturbances, since the eye is an unreliable instrument for judging the normalcy of skin. In contrast to biopsies, the use of non-invasive instruments also allows objective examination and follow-up of therapeutic effects without interfering with the studied skin area. Furthermore, the use of multiple devices facilitates the characterization of a pathological condition. In the following, only methods related to the author's study will be discussed in more detail.

Skin surface topography

The topography of the skin may reflect the development, assembly and functional activity of the entire epidermis and dermis.

Methods of measurements

The skin surface can be assessed by analysis of video images or photographs of the skin (Marshall 1983, Stone 1990), by analysis of shadows produced on a skin replica (Hayashi 1989, Miller 1987, Corcuff 1983, 1987, 1993, Murphy 1991) or by optical (Grove 1991) and mechanical (Cook 1980, 1982, 1985, Mignot 1987, Makki 1979, 1984, Murahata 1984, Monti 1989) profilometry of replicas. The typical use of image analysis for surface measurements is dependent on the interaction of an illuminating beam with the surface and the subsequent production of shadows. The shadows can then be selected and measured in different ways. The method appears to be less time-consuming than profilometry (Corcuff 1993).

Mechanical profilometry is based on the use of a profilometer whose fine tip follows the local variations in relief (Cook 1980, 1982, 1985, Mignot 1987, Makki 1979, 1984, Murahata 1984, Monti 1989). The topographical features of the surface induce vertical movements of the stylus, which are converted into electrical signals and subsequently digitized, yielding the profile of the cross-section of the surface. However, this technique cannot be applied directly on the skin, as the pressure that results from the contact with the diamond stylus is much too great. Thus, it is necessary to capture the surface details on a hard replica, which can then be analysed. The replication is a two-step process, requiring prior generation of a negative impression. High resolution silicone rubber has been used successfully for this purpose (Makki 1979, Murahata 1984, Cook 1980, 1982, 1985,

Monti 1989, Grove 1991, Nicholls 1978, Corcuff 1983), but because of its insufficient hardness, this silicone replica is then used as a cast for a hard replica. Various materials have been employed, such as polyethylene, epoxy resin, methacrylate-based material, and the styrene mounting medium DPX (Murahata 1984, Cook 1980, 1982, 1985, Nicholls 1978, Makki 1979, 1984).

Once a tracing of the skin surface has been obtained, a number of different methods can be used to quantify its characteristics. Most of the parameters have been borrowed from the metals industry, and a set of internationally recognized industrial standards have been specified according to the International Organization for Standardization (ISO). Parameters widely used to describe skin profiles fall into three categories (Mignot 1987, Cook 1980, 1982): (1) amplitude parameters that refer to vertical aspects of the profile, e.g. peak heights and furrow depths; (2) spacing parameters that refer to horizontal distances between features along the plane of the skin surface; and (3) so-called hybrid parameters that combine the size of vertical features with the distance between them. In the Experimental Procedures section some commonly used parameters are defined. It is difficult to say which of these parameters is best suited for measuring skin surface topography, since each describes a particular aspect of the profile. However, amplitude parameters and number of peaks appear to be useful in monitoring changes in the skin relief (Cook 1980, 1982, Marks 1975, Murahata 1984, Makki 1979, Mignot 1987, Grove 1991).

The profile created from a single slice through a specimen may not always be adequately representative of the topography of a particular skin (Makki 1979, 1984, Cook 1980, 1982). Multiple scans are therefore recommended to obtain reliable results (Makki 1979, Cook 1982). In addition to averaging results from multiple parallel scans, the anisotropy of the skin must be considered, since traces that run parallel and those that run perpendicular to the major furrows give different results (Murahata 1984, Cook 1980, 1982, 1985, Makki 1979). If several parallel profile traces are collected within a defined area of the replica, a three-dimensional reconstruction of the skin surface can be performed.

Applications of the method

Quantitative analyses of the surface topography have been performed to study the effects of ageing (Hayashi 1989, Mignot 1987, Berardesca 1991, Corcuff 1983, 1987, 1993), to investigate scaling disorders (Monti 1989, Murahata 1984, Cook 1985, Marks 1975, Gloor 1985, Murphy 1991), to characterize the

smoothing of plaques in localized scleroderma (Serup 1986), to classify malignant melanoma (Claridge 1992), and to examine the effects of drugs (Grove 1991, Monti 1989, Murphy 1991). The influence of moisturizers on the skin topography has been addressed in some studies (Batt 1986, 1988, Murahata 1984, Mignot 1987, Hayashi 1989, Nicholls 1978, Vilaplana 1992, Cook 1985). The roughness parameters and the distance between furrows/peaks have been used to describe changes in the hydration status (Cook 1982, 1985, Batt 1986, Murahata 1984, Mignot 1987, Batt 1988, Hayashi 1989). Dry skin tends to have a larger number of high peaks and a larger distance between the peaks than normal skin (Cook 1985). Hydration of normal skin has been reported both to decrease (Cook 1982, Batt 1986, 1988) and to increase (Murahata 1984) the roughness parameters. Cook (1982) found the distance between the peaks to be smaller after hydration. A single application of moisturizers has been found to decrease the roughness parameters and reduce the distance between the furrows during the first 2 hours (Mignot 1987). Hayashi (1989) reported that treatment of the skin for a longer period results in an increase in surface roughness and a reduced distance between the furrows. No change in the roughness but a decrease in the distance between the peaks was found after a 21-day treatment period in a study by Cook (1985).

Skin surface friction

Methods of measurement

The frictional properties of the skin surface reflect aspects of the microtopography of the skin surface and its elasticity. A variety of instrumental techniques have been employed to evaluate skin friction. Any technique that can simultaneously measure the normal load between the contracting surfaces and the force necessary to initiate motion can be used to determine the coefficient of friction (Wolfram 1989). The coefficient of friction in the skin may vary, however, depending on the load (Comaish 1971, Highley 1977, El-Shimi 1977), probably because of the elasticity of the skin. Equations describing the frictional behaviour between two materials suggest that for a constant load, softer material creates a larger contact area and thus higher friction than harder material (El-Shimi 1977).

Applications of the methods

In normal skin the frictional resistance varies in different anatomical regions, but not with age and sex (Cua 1990, Comaish 1971). A simple change in room

humidity can, by changing the skin hydration, modulate its frictional properties (Prall 1973, Sulzberger 1966, Naylor 1955). Hydration (Naylor 1955, Batt 1988, Comaish 1971, Nacht 1981, Highley 1977) and application of some moisturizers (Nacht 1981) have been shown to increase skin friction. Friction can be reduced by chemical modifications of skin proteins, rendering them less water soluble (Comaish 1973). Moreover, extreme wetness and dryness tend to decrease friction (Sulzberger 1966, El-Shimi 1977).

Skin dryness - water content

Methods of measurement

Most in vivo techniques for assessing skin hydration are based on the electrical properties of the skin, such as measurement of resistance, conductance, capacitance and impedance (Blichmann 1988, Lévêque 1983ab, Hashimoto-Kumasaka 1993, Ollmar 1994, Quattrone 1976, Potts 1986, Lodén 1994). Moreover, near-infrared spectroscopy has successfully been used to assess moisture in the skin (de Rigal 1993, Martin 1993). One commonly employed instrument for measuring skin capacitance is the Corneometer® (model CM 420 or 820, Courage & Khazaka, Cologne, Germany) (Triebkorn 1983, Thune 1989, Hashimoto-Kumasaka 1993, Berardesca 1990, Werner 1986, Blichmann 1988, Serup 1992ab). Another instrument is the Skicon100®, which measures skin conductance (Tagami 1982, Blichmann 1988).

The surface of the Corneometer probe works as a condenser and is influenced by changes in the dielectric constant of the material in contact with the probe. As water has by far the highest dielectric constant in the skin, an increase in water content will increase the capacitance (arbitrary units) in the Corneometer. This instrument has been reported to register hydration down to a depth of 0.1 mm (Blichmann 1988). One limitation of electrical measurements is that they only give qualitative information on differences in water content at poorly defined locations within the skin. Other drawbacks are that skin surface irregularities and other agents than water (e.g. urea and salts) may influence the readings of the skin hydration (Potts 1986, Lévêque 1983ab, Serup 1992a, Lodén 1994). Moreover, the skin hydration is easily changed by occlusion and contact with wet surfaces, and hence it is important that the probe does not alter the degree of hydration during the measurement.

Applications of the methods

Despite potential problems in determining skin hydration, studies have shown that measurement of skin capacitance (Serban 1983) and conductance (Lévêque 1987) appears useful in discriminating between different degrees of clinical dryness. Electrical measurements of dry skin in patients with atopic eczema (Werner 1986, Berardesca 1990, Thune 1989), psoriatic lesions (Takenouchi 1986, Serup 1987) and experimentally induced dry skin (Serban 1981) also show lower values than in normal skin, although some early studies with other techniques indicated increased hydration in dry atopic skin (Gloor 1981, Finlay 1980). The decreased ability of dry atopic and psoriatic skin to bind water has also been demonstrated by capacitance and conductance measurements (Tagami 1982, Thune 1989, Berardesca 1990).

In addition, electrical measurements have been used to study the hydrating effect of single applications of moisturizers to normal skin (Sindhvananda 1993, Tagami 1982, Serup 1992b, Frödin 1988, Blichmann 1989, Korstanje 1992). Application of petrolatum and dewaxed lanolin to the skin results in values indicating decreased skin hydration for some hours (Wepierre 1977). The reason for this is thought to be the high electrical resistivity of petrolatum, i.e. that petrolatum that has remained on the skin surface might depress the electrical response, so that the effect of these lipids is underestimated (Wepierre 1977).

Transepidermal water loss

Method of measurement

TEWL refers to the rate at which water migrates from the viable dermal and epidermal tissues through the layers of the stratum corneum to the external environment. It is a sensitive indicator of the integrity of the stratum corneum and measurement of TEWL is therefore used in many laboratories for studying skin water barrier function. TEWL can be measured conveniently with an Evaporimeter (ServoMed, Kinna, Sweden) (Nilsson 1977). Important factors to consider during TEWL measurements are room temperature and ambient humidity (Lévêque 1989, Pinnagoda 1990, Potts 1986). Sweating should be avoided and a room temperature of 20-22°C is therefore recommended (Pinnagoda 1990). Furthermore, air convection in the room may disturb the readings and some form of draught shield may be useful (Pinnagoda 1990). The rest time before TEWL readings also has to be considered (van Sam 1994).

Another important finding is that other volatile agents than water might influence the readings if measurements are made immediately after application of moisturizers (Morrison 1992). Critical assessments of the validity of TEWL techniques can be found in recent reviews (Potts 1986, Lévêque 1989), and guidelines for measurement of TEWL were recently published by the standardization group of the European Contact Dermatitis Society (Pinnagoda 1990).

Application of the method

TEWL measurements have proved useful in detecting differences in skin permeability between individuals (Blichmann 1987) and between body regions (Rougier 1988, van Sam 1994). The individual TEWL values appear to be constant, since repeated measurements at a 1-2 month interval show highly reproducible values (Oestmann 1993). With these measurements it has also been shown that dry, scaly skin in some conditions is more permeable than normal skin (Denda 1992, 1994, Werner 1985, Thune 1989, Finlay 1980, Motta 1994, Serup 1987, Takenouchi 1986, Prottey 1976).

Furthermore, it has been proposed that TEWL might serve as an indicator of the permeability of the skin to topically applied substances, such as hydrocortisone and benzoic acid (Aalto 1993, Dupuis 1986). High basal values also seem to indicate an increase in skin susceptibility to irritant stimuli (Tupker 1989, Agner 1991). Various authors have studied TEWL upon application of soaps and detergent bars (van der Valk 1984a) and various surfactants (Agner 1989, 1990, van der Valk 1984b). Measurement of TEWL can also be used to monitor irritation and barrier repair as a function of time (Hannuksela 1992, Holleran 1991, Grubauer 1987, 1989a, Ghadially 1992, Tolleson 1993b), and for comparing different degrees of irritation observed visually as related to some kind of barrier damage (van der Valk 1984b, Agner 1990).

Cutaneous blood flow in response to irritant stimuli

Method of measurement

The intensity of the skin response to a topically applied irritant is determined by the effectiveness of the permeability barrier and by the inflammatory potential of the irritative substance. Measurements of the cutaneous microcirculation can be used to study this skin response. An easily handled method for non-invasive recording of microvascular blood flow is laser Doppler flowmetry (Tenland 1982, Bircher 1994).

The measurement is based on the Doppler principle for detection of particle movement, in this case using light scattered from erythrocytes (*Tenland 1982*). A number of studies have been performed to correlate visual grading of skin irritant reactions with cutaneous blood flow values obtained with a laser Doppler flowmeter (*Nilsson 1982, Staberg 1984, Agner 1990, Serup 1990*). The results indicate that this instrument is useful in discriminating between negative and positive reactions but fails to quantify strongly positive reactions. It is possible that in advanced reactions oedema compresses the vasculature (*Serup 1984, 1990*) or that more pronounced vasodilatation does not increase the Doppler shift (*Agner 1990*). In some cases the instrument may also be more sensitive than the naked eye (*Nilsson 1982*). Guidelines for measurement of cutaneous blood flow have recently been published (*Bircher 1994*).

Application of the method

Clinical assessment of the degree of irritation caused by chemical agents applied to the skin remains subjective. On the other hand, quantitative readings of patch test reactions using measurements of cutaneous blood flow make it possible to compare results obtained in different laboratories and by different investigators (*Nilsson 1982, Agner 1990, Serup 1990*). Laser Doppler measurements have also been used to study effects on barrier properties, such as the influence of hydration on the penetration of hexyl nicotinate, a substance with a local vasodilatory effect (*Ryatt 1988*). Furthermore, measurement of cutaneous blood flow can be used to study some physiopathological data in skin diseases such as Raynaud's phenomenon, scleroderma, psoriasis and burns (reviewed in *de Lacharrière 1989, Dittmar 1989*).

Sensory evaluation of skin care products

A lot of art and instinct is used in the formulation and evaluation of skin care products. Often the development consists of minor improvements in product performance which require carefully conducted experiments in order to provide definite information. Instrumental evaluation of the produced skin changes provides a possibility of obtaining objective and reproducible data. However, changes in instrumental readings may not always be perceived by the users of the product. Hence, it may be appropriate

to add sensory evaluation to those studies whose results will be used for making claims about the product. Furthermore, to introduce new quantitative data for product performance it is necessary that the sensory analysis and the instrumental data correlate closely.

Traditionally, food scientists and manufacturers have taken the lead in developing sensory testing procedures. Some key issues in the evaluation of sensory attributes and acceptance of cosmetics will be discussed briefly. More information is to be found elsewhere (*Civille 1991, Stone 1985, Moskowitz 1984*).

Acceptance testing

A good understanding of consumer perceptions, moods and changing fashions is of great importance in developing and positioning cosmetic products. For instance, to obtain maximum acceptability a moisturizer should have a pleasant odour and tactile feeling on application, otherwise it will not be used, even if it has the desired biological effects. However, it may not be obvious to the producer what kinds of sensory attributes are important to the users of a particular product. Information about the current or potential users may be obtained by acceptance testing, also referred to as consumer or preference testing. In this type of testing, the degree of liking of or preference for a product based is evaluated on the basis of its sensory properties. To avoid influences of artwork on the evaluation, the product should be presented to the test subject in a blind fashion. Hence, products tested under actual use conditions in the home should be packaged in plain white containers labelled only with the product code, usage instructions, expiry date and reference to someone responsible for the test, preferably not revealing the marketing company. More simple labelling can be used if the product is tested in a laboratory or at some central location (e.g. a shopping centre).

The subjects participating in acceptance testing should be carefully selected. The panel of consumers should be chosen among current or potential users of the product type in question, if the aim of the study is to optimize the sensory attributes of a particular product. It is of little or no value to assess the reactions of consumers to products that they would never use. However, the study might also be undertaken to obtain information on the acceptance of a particular product in different populations in order more efficiently to be able to design the marketing strategy.

There are numerous procedures for ascertaining whether or not a person likes a particular product, and to what degree. The person can simply categorize the feeling into like or dislike, or scale the degree of liking. However, it is not appropriate to include assessment of product sensory attributes in the same study, although this is often done.

Sensory attributes

Skin care products are complex formulations which present the user with a variety of sensory stimuli. As a basis for a descriptive analysis of the sensory stimuli exerted by a product, a set of terms or words that appropriately describe the sensory attributes is constructed. From a product development viewpoint, it is essential to focus on those product variables that are perceived as important. In Table 3 some examples of visible and tactile characteristics of creams are given. Subjects who agree to the meaning of the selected words are then able to quantify the intensity of these stimuli. Hence, in quantitative descriptive analyses, trained panellists replace measuring instruments, and as with instruments, considerations concerning the reliability, validity, detection level etc., need to be addressed.

Discrimination testing is a class of test procedures that often precedes descriptive analysis. The panellists, who can be either trained experts or consumers, are asked to discriminate between two or more products. If the test method is organized as a triangle test, three samples are coded and the panellists are asked to determine which two samples are the same or which sample is different from the other two. On the basis of a perceived difference, for instance, between a market leader and a new prototype formulation, the testing can proceed with a descriptive analysis of the intensity of the product attributes. Furthermore, discrimination testing increases the possibility of detecting changes in product quality due to storage, modification of the composition, or changes in the manufacturing instructions.

Table 3. Examples of attributes of a cream that can be rated by panellists.

Visible characteristics	Colour, colour intensity, shine, homogeneity, gloss.
Tactile characteristics	Spreadability, slipperiness, oiliness, wetness, stickiness, tackiness, dragginess, smoothness, richness, waxiness, flakiness, absorbancy, residue.

Scales of perception

A rational basis for decisions about the products is the scaling of the perceived stimuli and subsequent statistical analysis. Many different scales exist, each with different properties. For instance, products can be ranked according to the degree of liking or to the intensity of a particular sensory stimulus. Category scales represent interval scales that can be used in both acceptance and product testing, as well as in clinical dermatology (Table 4). The scale comprises a fixed set of categories that describe each scale point. The scale sensitivity can be augmented by increasing the number of categories or by using a variation of the interval scale known as the "line scale" or visual analogue scale. When using visual analogue scales, panellists mark their rating on a continuous line with end-points at its extremes. The key benefit of the use of the line lies in its ability to diminish the variation in the panellists' rating and in the fact that it allows the panellists to use the scale in a way they find comfortable.

Table 4. Examples of fixed-point category scales.

Hedonic scale	Intensity of a sensory attribute	Therapeutic response
Dislikes a lot	None	None
Dislikes a little	Slight	Little
Neither likes nor dislikes	Moderate	Moderate
Likes a little	Strong	Marked
Likes a lot	Extreme	

Skin care products

The main purpose of a skin care product is to support the natural skin functions. Since the skin is a powerful organ of non-vocal communication, the use of colour cosmetics enhances our communicative skills. It has been claimed that the use of cosmetics improves physical attractiveness and personality traits (*Graham 1980*). Not only colour cosmetics, but the vast majority of the available skin care products are able to modify the appearance or odour of the users. Soaps, cleansers and shampoos remove dirt, and in combination with antiperspirants, deodorants, aftershaves and perfumes, they improve body odour. Sunscreens protect the skin against UV radiation. Emollients and moisturizers are used to break the dry skin cycle and to maintain the smoothness of the skin. The term "emollient" implies (from the Latin derivation) a material designed to soften the skin, and usually we mean a material that "smooths" the surface to the touch and makes it look smoother to the eye. The term "moisturizer" is often used synonymously with emollient, but moisturizers often contain humectants in order to hydrate the stratum corneum. More about the definitions of emollients and moisturizers can be found in an article by *Gabard (1994)*.

When developing skin care products, it is important to consider the chemical, physical and dermatological characteristics of the formulation, as well as those of the individual ingredients. More than 6,100 cosmetic ingredients are used to formulate cosmetic products and they are listed in the International Cosmetic Ingredient Dictionary (*Wenninger 1993*). The formulation functions of common ingredients have been compiled by *Barry (1983)*.

The major unwanted effects of cosmetics include irritation (objective and/or subjective), contact allergy, photosensitivity and contact urticaria (*Cronin 1980, Nater 1983, De Groot 1988abc, Berne 1994*). These effects are not dealt with in this thesis.

Effect of cleansing products

Cleansing products are composed of soap and/or synthetic surfactants. Soap is the general name for a surface active salt of a fatty acid. Sometimes the cleansers are formulated with the addition of inert granules, e.g. polyethylene, to enhance the cleansing effect, or with antimicrobials for deodorisation and

microbiological effectiveness. The capacity of cleansers to remove dirt, desquamating cells, cutaneous secretions and pathogenic microorganisms may also affect the properties of the skin. Irritation may be evoked by modification of the stratum corneum and/or by penetration of surfactants into the viable skin (*Lodén 1990, Hunziker 1992, Seidenari 1994, Agner 1989,1990, Fulmer 1986, Ollmar 1994, Serban 1981, Halkier-Sørensen 1993, Denda 1994*). The granules in abrasive cleansers - scrub creams - are supposed to mechanically remove the loosely bound outermost layer of the stratum corneum (*Garber 1976*), and users often report an increased smoothness of the skin after scrubbing, which is probably due to this gentle polishing.

Effect of moisturizers

Application of moisturizers to the skin induces tactile and visual changes of the skin surface. For product attributes, not only is the ratio between oil and water important, but also the type of oil and the amount and type of other ingredients (emulsifiers, humectants, etc.). The combination of substances influences the initial feel of the product, its spreading behaviour on the skin, whether and how fast it is absorbed, and how the skin feels after its use.

Moisturizers may modify the physical and chemical nature of the surface, to one that is smooth, soft and pliable. Smoothing of the surface can be observed immediately after application of a moisturizer as a result of the filling of spaces between partially desquamated skin flakes (*Nicholls 1978, Garber 1976*). Besides mixing with material already present on the surface, topically applied substances may disappear from the skin by contact with other surfaces, evaporation (*Blichmann 1989, Rietschel 1978*), absorption (*Lodén 1990, 1994*) and metabolism (*Hansen 1991, Lodén 1985, Wertz 1990*). The changes in the tactility of the skin surface are not only due to the amount of moisturizing substances on the top layer of the stratum corneum. The main goal in using moisturizers is, namely, to increase the elasticity of the stratum corneum by increasing its moisture content. Traditionally this is believed to be achieved by two different means: (a) by occlusion of the skin surface, and (b) by introduction of humectants which are able to maintain the moisture in the stratum corneum. Water in the applied products is believed not to contribute to the increase in hydration, since water easily evaporates from the surface (*Blichmann 1989, Rietschel 1978*).

Lipids and the skin

Occlusion of the surface implies a simple reduction of the loss of water from the outside of the skin. Common occlusive substances in moisturizers are lipids, for instance petrolatum, beeswax, lanolin and various oils. Although they prevent water loss (*Lieb 1988*), their effect may be reduced when combined with other ingredients in skin care products (*Wepierre 1982, Choudhury 1985*). These lipids have long been considered to exert their effects on the skin solely by forming an inert, epicutaneous, occlusive membrane. They are therefore incorporated into formulations on the basis of their technical and sensory properties rather than on their possible impact on the epidermis. However, recent studies indicate that topically applied lipids penetrate the skin (*Moloney 1988, Wertz 1990, Dewsbury 1989, Escobar 1992, Tolleson 1993ab*). Lipids may also enter into epidermal metabolism and modify endogenous epidermal lipids (*Wertz 1990, Rawlings 1994*). A recent study has shown that application of a vegetable oil containing linoleic acid changes the ceramide levels in normal human stratum corneum (*Rawlings 1994*). The smaller amounts of one ceramide found in the stratum corneum in winter were restored to the larger amounts found in summer months (*Rawlings 1994*).

Lipids may interfere with the lipid layer around the corneocytes, and thereby artificially retain the moisture content in the corneocytes and/or restore an impaired permeability barrier function (*Ghadially 1992, Imokawa 1985, 1986, 1989, 1991b*). Lipids in bland ointment bases have also been found to have an antimitotic effect on the epidermis of stripped dorsal skin of hairless mice, and occlusion was believed not to be the entire explanation (*Tree 1975*).

In clinical studies it has been found that the use of moisturizers without any known pharmacologically active ingredients improve several dry skin conditions. Irritant contact dermatitis, caused by exposure to surfactant solutions, improves on treatment with emollients (*Hannuksela 1992, Halkier-Sørensen 1993*). Likewise, the symptoms of dry skin conditions, such as ichthyosis, asteatosis, dry chapped hands and heels, atopic eczema and atopic dry skin, are relieved by treatment with moisturizers (*Grice 1973, Pope 1972, Frithz 1983, Middleton 1978, Dunlap 1984, Swanbeck 1968, Dahl 1983, Rosten 1970, GPR 1973, Siskin 1993, Vilaplana 1992, Fredriksson 1975, Halkier-Sørensen 1993, Serban 1983, Serup 1992a, Rattner 1943*). In studies involving measurements of TEWL, this water loss decreased in association with improvement of the dryness in some (*Grice 1973, Serban 1983, Serup 1992a*), but not all studies (*Halkier-Sørensen 1993*). The compound(s)

responsible for the effects was not identified, although in some studies humectants were found to enhance the improvement (*Grice 1973, Pope 1972, Frithz 1983, Middleton 1978, Dunlap 1984, Rattner 1943*).

Attempts have been made in some studies to identify the beneficial ingredients. In an experimental model of sodium laurate-irritated rat skin, it was suggested that linoleic acid in sunflower seed oil reduced the abnormally high rates of TEWL (*Prottey 1976*). In contrast to these findings, an inverse relationship was found between recovery of normal TEWL and the amount of sunflower seed oil in emulsions used for treatment of sodium lauryl sulphate (SLS)-induced irritation in man (*Blanken 1989*). Although unsaturated fatty acids are necessary for the maintenance of the epidermal permeability barrier, treatment of SLS-damaged skin with linoleic acid did not appear to improve the water barrier function (*Blanken 1989*). This could possibly be anticipated, since no decrease in the amount of ceramides containing linoleic acid occurs on repeated exposure to SLS, although changes in the distribution of certain ceramide species have been found (*Fulmer 1986*). However, topical treatment with linoleic acid or sunflower seed oil, rich in linoleic acid, readily reverses the syndrome of EFA deficiency (*Press 1974, Feingold 1986, Prottey 1976*).

Topical (*Dewsbury 1989, Escobar 1992*) as well as oral (*Bittiner 1988*) treatment with fish oils rich in omega-3 fatty acid is claimed to be effective against psoriasis, although this has been questioned (*Henneicke-von Zepelin 1993, Bjørneboe 1988, Gupta 1990*). Recently it was also reported that dietary supplementation with fish oil (*Orengo 1992, Rhodes 1994*) and purified ethyl ester of eicosapentaenoic acid (20:5, n-3) from fish oil (*Danno 1993*) has anti-inflammatory effects on UVB-induced acute inflammation. Evening primrose oil, a vegetable oil rich in gamma linolenic acid, GLA, (a fatty acid of the omega-6 family), has been found to be effective against atopic dermatitis when administered orally (*Lovell 1981, Wright 1982*). However, this has not been confirmed in other studies (*Skogh 1986, Bamford 1985*). Topical application of another oil, borage oil, is claimed to have good effect against infantile seborrhoeic dermatitis by virtue of its high content of GLA (*Tolleson 1993ab*). The biochemical mechanisms of the possible therapeutic effects remain unclear, but studies indicate that the polyunsaturated fatty acids in the oils can be transformed enzymatically by the epidermis into "putative" anti-inflammatory products (*Miller 1991*). The enzyme δ -6-desaturase, which converts linoleic acid into GLA, might also play a role, since it has been suggested that it may be

impaired in atopic eczema and seborrhoeic dermatitis (Tolleson 1993a, Manku 1984).

Recent studies have also shown that topically applied lipids may interfere with the lipid synthetic activity of the skin (Ghadially 1992, Man 1993). Petrolatum has been found to be absorbed into delipidized skin and to accelerate barrier recovery (Ghadially 1992). However, applications of ceramides, linoleic acid and a variety of other fatty acids alone have been reported to actually delay barrier recovery in acetone-treated murine skin, despite the fact that these lipids are required for barrier homeostasis (Man 1993). Moreover, two-component mixtures of fatty acid plus ceramide, cholesterol plus fatty acid, or cholesterol plus ceramide delayed barrier recovery. The only treatments that allowed normal barrier recovery were applications of complete mixtures of ceramide, fatty acid and cholesterol, or pure cholesterol (Man 1993).

Humectants and the skin

Humectants widely used in moisturizers are PCA, lactic acid, urea, glycerine and sorbitol. The water binding capacity of the sodium salts of lactic acid and PCA appears to be higher than that of glycerine and sorbitol (Takahashi 1984, Rieger 1974). Urea also has strong osmotic activity (Hellgren 1974, Swanbeck 1978). However, which of these substances most efficiently increases the skin hydration is not known. Besides differences in water binding capacity, their absorption into the skin is probably important for the effect. The amount of urea (Wellner 1993, Lodén 1994) and glycerine (Batt 1986) absorbed into normal stratum corneum was recently determined using a simple tape-stripping technique.

As may be anticipated, the water-holding capacity of normal stratum corneum and of scales from psoriatic and ichthyotic patients is substantially increased after treatment with urea and glycerine preparations (Batt 1988, Swanbeck 1968, Grice 1973, Tagami 1982). Likewise, PCA attracts water and increases the degree of hydration of solvent-damaged guinea pig footpad corneum (Middleton 1978). Furthermore, clinical studies have shown that regular use of moisturizers containing humectants more efficiently than moisturizers without humectants relieve the symptoms of dry skin conditions, such as ichthyosis, asteatosis, dry chapped hands and heels, atopic eczema and atopic dry skin (Grice 1973, Pope 1972, Frithz 1983, Middleton 1978, Dunlap 1984, Dahl 1983, Rattner 1943). Moreover, treatment of dry skin with moisturizers containing humectants, such as urea or glycerine, has been shown to restore the impaired permeability barrier to water (Grice 1973,

Serban 1983, Serup 1992a). However, urea did not promote normalization of TEWL in an experimental model on surfactant-induced irritation in man (van Neste 1988). In another study on dry skin in atopic and non-atopic individuals, a moisturizer containing ammonium lactate as humectant had no effect on TEWL, although the clinical appearance improved (Vilaplana 1992).

Another proposed effect of moisturizers is that they might influence the crystalline arrangement of the bilayer lipids (Mattai 1993). In dry skin the proportion of lipids in the solid state may be increased, and putative moisturizers may then help to maintain the lipids in a liquid crystalline state at low relative humidity (Mattai 1993, Froebe 1990). Glycerine has been shown to interact with model lipids to maintain the liquid crystalline state even at low relative humidity (Froebe 1990). It has also been proposed that glycerine may aid the digestion of the superficial desmosomes in subjects with dry skin and thereby ameliorate dry flaky skin (Rawlings 1993a). Furthermore, α -hydroxy acids, such as lactic acid, might be useful in moisturizers because of their influence on stratum corneum elasticity (Alderson 1984, Takahashi 1985, Hall 1986, Hagan 1993).

Influence on the permeability barrier function of normal skin

Despite the widespread use of moisturizers, scant attention has been paid to their influence on the permeability barrier of normal skin. It may be anticipated that the use of moisturizers will increase the permeability, since hydration of normal skin is known to reduce its diffusional resistance (Ryatt 1998, Cooper 1976, Blank 1984). In vitro experiments on stratum corneum have also shown that humectants increase TEWL (Rieger 1974, Lieb 1988). However, in studies on healthy volunteers no change in TEWL was observed, although the applied moisturizer appeared to increase the skin hydration significantly (Frödin 1988, Bimczok 1992, Serup 1989). So far, there appear to have been no investigations on the possible effects of moisturizers on skin susceptibility to irritants in the environment. However, the results of one study indicated that moisturizers might prevent irritant contact dermatitis, since a lower degree of irritation was noted when moisturizers were applied after exposure to dishwashing detergent than if no moisturizer was used (Hannuksela 1992). Whether this beneficial effect of the moisturizers was therapeutic or preventive was not clearly elucidated.

Protective creams, also called barrier creams or invisible gloves, are expected to form an impermeable

film that can prevent noxious substances from coming into contact with the skin. To what extent a cream really can prevent this contact is questionable (Lodén 1986a). Some creams may delay the contact, depending on their composition and layer thickness (Boman 1982, Wahlberg 1972). Other creams, however, may enhance the penetration of the hazardous substance (Fischer 1983).

Concern has been expressed about the use of urea in moisturizers, with reference to the risk of reducing the chemical barrier function of the skin to toxic substances (Hellgren 1974). That urea can increase skin permeability has also been demonstrated in several studies, where urea was found to be an efficient accelerant for the penetration of several substances (Wohlrab 1984, 1989, 1990, Allenby 1969, Kim 1993, Beustall 1986). However, not all studies support the general belief that urea is an effective penetration promoter (Wahlberg 1973, Stüttgen 1989, Lippold 1990), and studies on dry (Serup 1992a) or ichthyotic (Grice 1973) skin have shown that urea-containing moisturizers decrease TEWL. In addition, other humectants, e.g. sodium lactate, sodium-PCA and sorbitol, have been reported to actually reduce the penetration of benzyl nicotinate (Lippold 1990).

AIMS OF THE INVESTIGATION

In the present investigation non-invasive biophysical methods were used with the following aims:

- to further characterize the surface features and barrier function of dry atopic skin and normal skin;
- to study the effects of treatment with skin care products on the topography, friction, hydration, permeability of normal skin and on its susceptibility to an irritant stimulus (SLS);
- to illustrate the hydrating mechanisms of moisturizers;
- to study the impact of different lipids on surfactant-induced irritation.

EXPERIMENTAL PROCEDURES

Methods

Determination of topography (I and III)

The surface features of dry atopic skin (I) and of normal skin treated with a scrub cream (III) were

compared with those of normal skin. The skin surface topography was transferred onto a hard material in a two-step process. A negative replica of the skin surface was generated using a silicone-based dental impression material and this replica was then used as a cast for a hard methacrylate-based positive replica. The material was properly mixed and care was taken not to produce any air-bubbles in the material. As the roughness values are dependent on the orientation of the replicas, the replicas were marked to facilitate the orientation during the analyses (Makki 1979, Cook 1982, 1985). In our studies, the traces were run perpendicular to the major furrows.

The replicas were analysed using a Perthometer C5D together with a precision traversing table developed at the Department of Production Engineering, Chalmers, Sweden (Bengtsson 1991). An IBM-compatible XT microcomputer was used to control the table and to collect and store the data. The analogue signals of the vertical displacements were digitized with an A/D converter. From each replica, 75 parallel profile traces (interval 40 μm) were collected within an area of 6.0 x 3.0 mm. Each profile trace consisted of 1,024 points, i.e. altogether 76,800 points were collected. These data could then be used to calculate texture parameters and to build up a three-dimensional reconstruction of the skin microtopography. Prior to the analysis, a mean plane was fitted to the data using the least square method.

Surface texture parameters

The values for the parameters were first computed for each individual profile trace, whereafter the mean values of the 75 traces on each replica were calculated. Parameters describing the texture of the surface can be grouped into two categories: amplitude parameters (R_a , R_q , and R_y) and shape parameters (R_{sk} , R_k , R_n , λ_a , and Δ_a). Amplitude parameters are measures of the vertical characteristics of the surface deviations, whereas shape parameters are a combination of the horizontal and vertical characteristics of the surface. The parameters are defined according to International Standards ISO 4287/1 (published by the International Organization for Standardization):

- R_a (μm): The most widely used parameter for roughness. This parameter comprises the arithmetical mean of the absolute values of the profile departures of the mean line within the sampling length.
- R_q (μm): The root-mean-square parameter corresponding to R_a .
- R_y (μm): The maximum peak-to-valley height of the entire surface.

- R_{sk} , skewness: The measure of the symmetry of the distribution density of profile about the mean line. The value is an indicator of the cumulative width of peaks in relation to the cumulative width of the furrows, i.e., of the asymmetry of the height distribution in relation to the mean line. Skewness is negative when the relief is mainly formed of broad peaks (plateaus) and positive when the furrows mainly have horizontal bases. This parameter is unitless.
- R_k , kurtosis: A measure of the sharpness of the surface profile. Kurtosis = 3 for a Gaussian distribution. Kurtosis <3 indicates that the base of the distribution curve is wider than a Gaussian curve. This parameter is also unitless.
- R_n : The number of peaks per cm about the mean line.
- λ_a , wavelength: The average measure of the spacings between local peaks and valleys, taking into account their relative amplitudes and individual spatial frequencies.
- Δ_a : The arithmetical mean slope in radians of the profile.
- T_p : The ratio of the profile bearing length to the sampling length, i.e. the sum of the section lengths obtained by cutting the profile peaks by a line parallel to the mean line, within the sampling length and at a given section level of R_y (%). A bearing length ratio at different depths was calculated from the sum of the profile bearing lengths divided by the sum of the sampling lengths. Bearing area ratio curves were established when dry atopic skin was compared with normal skin.

Determination of friction (II and IV)

Instrumental measurement (II, IV)

The frictional properties of dry atopic skin (II) and of normal skin treated with different moisturizers (IV) were measured with an instrument developed at the School of Dentistry, Malmö, Sweden (Olsson 1994, Henricsson 1990). The instrument is a new device constructed with the purpose of objectively measuring oral mucosal surface friction (Henricsson 1990). The friction instrument consists of a computerized probe with an oscillating steel plate, diameter 0.6 cm (Olsson 1994). The probe was applied perpendicularly to the surface and friction values (arbitrary units) were obtained by measurement at an axial load of about 0.1N. At this low load no visible twisting or wrinkling of the skin occurred. The topographical features of the steel plate were determined by profilometry (Table 5) according to the previously described method. The

results showed that the surface of the probe was much smoother than normal skin (cf. Tables 5 and 6).

Table 5. Topography of the steel probe used for friction measurements.

Roughness parameter	Value
R_a (μm)	0.14
R_q (μm)	0.18
R_y (μm)	2.87
λ_a (wavelength, μm)	22.7
Δ_a (radians)	0.04
R_{sk}	-0.71
R_k	5.49

Sensory analysis (IV)

Panellists trained in sensory evaluation of creams were asked to evaluate the friction following application of moisturizers. The perceived degree of friction was marked on a 15-cm visual analogue scale, where the end-points of the line reflect a continuum from very low to very high friction. Before application of the creams, the panellists were "calibrated" by being asked to estimate the basal level of the skin friction. They were instructed that the basal level was anchored 6 cm from the left end of the line, and that the scale values emanated in two directions from this normalized point.

Acceptance testing (IV)

The subjective attitudes to the perceived skin feel after application of three moisturizers were evaluated in a consumer test. The results were marked on an analogue scale, where the left end reflected very unpleasant friction and the right very pleasant friction. The subjects were also asked to mark on a hedonic scale the term that best represented their attitude about the feeling, among the following: much too slippery (1), too slippery (2), pleasant (3), too stiff (4) and much too stiff and tacky (5).

Determination of barrier function and skin susceptibility to irritants (III, VII)

To a certain degree, TEWL gives an indication of the skin permeability. In order to further increase our knowledge on the skin integrity, we developed a method in which we exposed the skin to an irritating detergent, SLS. SLS is commonly used in experimental dermatology. It penetrates human skin (Lodén 1990) and induces significant changes in

cytokine concentrations in the afferent skin lymph (Hunziker 1992) and various visible and invisible changes which can be detected by non-invasive bioengineering techniques (Agner 1989, 1990, Ollmar 1994, Seidenari 1994). It has been shown that SLS of different qualities have different skin-irritating abilities (Agner 1989). In the present work, aqueous solutions of SLS according to the European Pharmacopoeia (Ph. Eur.) were used in the experiments. Fifty microlitres was pipetted into large aluminium Finn Chambers® (diameter 12 mm, Epitest Oy, Finland) containing one layer of filter paper. The chamber was attached to the skin for 7 h with adhesive tape (Scanpor®; Norgeplaster, Norway).

Determination of clinical dryness and degree of irritation (II, III, VII, VIII)

Clinical dryness (II)

The appearance of the dry non-eczematous skin in patients with atopic dermatitis was graded clinically as follows: normal or smooth (-), rough with slight scaling (+), rough with scaling (++) and rough with marked scaling (+++).

Clinical signs of irritation (III, VII, VIII)

The skin response following exposure to SLS was examined visually. The evaluation was made without access to the application charts. In study III the reactions were ranked according to the degree of irritation and in studies VII and VIII the degree of irritation was scored according to the following scale: no reaction (0), barely perceptible very weak spotty erythema (0.5), slight erythema, either spotty or diffuse (1), moderate erythema (2) and intense erythema, with infiltration and possible vesicles (3).

Determination of electrical capacitance (II, V, VII)

The electrical capacitance of the skin was determined with a Corneometer® CM 420 or CM 820 (Courage & Khazaka GmbH, Köln, Germany).

Determination of water evaporation from the skin (II, III, VI, VII, VIII)

Measurements of the release of excess water from the skin (VI) and the passage of water through the epidermis (II, III, VII, VIII) were performed using the Evaporimeter EP1 (Servomed, Kinna, Sweden). The probe for the measurement was equipped with a screen

and grid to reduce air convection (Pinnagoda 1990). The room temperature was between 20 and 22°C. Noise in the room and talking during the recordings were restricted.

Determination of cutaneous blood flow (III, VII, VIII)

A Perflux Pf1 laser Doppler flowmeter (Perimed, Stockholm, Sweden) (Tenland 1982) was used for measuring superficial blood flow. The instrument was equipped with a special multifibre probe (PF 113 Integrating probe, Perimed) which has seven fibre triplets instead of one - one being in the middle and six around it, forming a circle 8 mm in diameter, in the probe head. Thus, the blood flow value is the mean of the seven spots, which reduces variation due to spotty erythema. The probe was attached to the skin with a standard probe holder without pressure and with use of double adhesive tape.

Recordings

The output signals from TEWL and blood flow measurements were recorded with a pen recorder (Servogor 120, BBC) until equilibrium was reached, usually within 1-2 min. This revealed whether the test subject was in a relaxed and comfortable state or not. The value at equilibrium was used for the calculations.

Volunteers and study design

Determination of topography (I, III)

Atopic skin (I)

The surface features of dry atopic skin (I) were compared with those of normal skin after the topography had been captured on replicas. The replicas were taken on the back (10 females, aged 18-40 years, mean 29). All atopic subjects, as assessed by the criteria of Hanifin & Rajka (1980), had dry skin in that area, defined as a rough, finely scaling, non-inflamed skin surface. Ten females (aged 18-40 years, mean 29) without any kind of atopy or dry skin served as controls.

Scrubbed skin (III)

In study II, for evaluating the effects of the scrub cream, 11 female subjects (aged 22-59 years, mean 42) without skin diseases participated. Three replicas were taken on the volar aspect of the forearm. The first replica was taken prior to the treatments and the

second one after washing the surface with liquid soap and water and allowing the skin to dry. The third replica was generated after gentle scrubbing of the skin with a scrub cream (ACO Scrub Cream, ACO Hud AB, Sweden) for 20-30 s, rinsing with water, blotting with soft paper, and allowing the skin to dry.

Determination of skin removal by the use of a scrub cream (III)

In study III the degree of skin removal after using a scrub cream was investigated in 9 females without skin diseases (mean age 36 years). In this study a new concept was used. The amount of skin removed by scrubbing was compared with that removed by successive stripping of the skin with tape. Five areas on the volar aspect of the forearm were used. One area was gently scrubbed for 30 s, one area was left untreated and the remaining three areas were stripped with Scotch™ invisible tape 3, 6, or 12 times. The treatments were randomly allocated to the five areas. Following the treatments, all five areas were exposed to 50 µl of 8% SLS in distilled water for 7 h. Fourteen hours after removal of the patches, the skin reactions were evaluated using a laser Doppler flowmeter and an Evaporimeter. The areas were examined visually and ranked according to the degree of irritation. All assessments were performed in a blind manner without access to the application chart.

Determination of friction (II, IV)

Atopic skin (II)

Skin friction was measured at three body sites in 11 patients (mean age 29 years) with atopic dermatitis, diagnosed according to the criteria of Hanifin & Rajka (1980), and 15 persons (mean age 27 years) without any skin diseases (II). The atopic patients had dry-looking skin, but no eczema, on one or more of the following studied areas: the dorsum of the hand, the lower back and the volar aspect of the forearm. Besides measurements of friction, the skin capacitance and TEWL were recorded. Prior to the measurements the dryness of the skin was graded clinically. The study took place during one week in October.

Influence of moisturizers - instrumental assessment (IV)

The frictional behaviour of five moisturizers (A-E) was studied in seven volunteers (mean age 39 years) (IV). Before application of the creams, the basal friction value was recorded. The products were dispensed by a volumetric syringe to the inner forearm

of the volunteers. The application rate was about 5 mg/cm². The friction was measured immediately after application of the product and then every fifth minute up to 30 minutes. Between each measurement the probe was cleaned.

Influence of moisturizers - sensory evaluation (IV)

In a double-blind study comprising 11 experienced test persons, the subjectively perceived skin friction after application of five moisturizing creams (A-E) was investigated. The mean age of the persons was 46 years (range 41-50).

The products were submitted to the panellists in random order. They were dispensed from coded volumetric syringes to the inner surface of the panellist's forearm. The application rate was 5 mg/cm². The panel members were asked to spread the product over and into the skin with their fingertips. The ease with which the fingertip could be moved over the surface during 10 s was evaluated as friction. The sensory magnitude of friction was estimated on application of the product and every fifth minute thereafter during a period of 15 min. The fingertips were cleaned between each application by wiping with soft paper.

Acceptance testing (IV)

The subjective attitudes to the perceived skin feel after application of three moisturizers (A, B and E) were evaluated in a consumer test. Fifteen users (mean age 41 years, range 29-57) of skin care products were selected among individuals with no apparent connection with any of the tested creams. The products were dispensed from coded volumetric syringes to the inner surface of the forearm in random order. The subjects were asked to spread the products on the skin surface and assess the degree of liking during spreading. Fifteen minutes later they were again asked to assess the degree of liking of the skin resistance, according to the evaluation protocol.

Determination of hydration induced by moisturizers (V, VI)

Measurement of skin capacitance (V)

The change in skin capacitance on the volar aspect of the forearm following application of four skin care products was studied in 12 females (mean age 39 years) with no clinical signs of skin diseases or dry skin (V). Using a micropipette, 5 mg/cm² of the three cream emulsions and 2.5 mg/cm² of petrolatum were dispensed to four areas on each arm. Larger doses of the cream emulsions were applied, since they contained

more than 50% of volatile ingredients, mainly water. A fifth area on each arm served as control and was left untreated. The products were randomly allocated to the five sites. Before application, the skin was cleaned with soft paper impregnated with dehydrated diethyl ether. The electrical capacitance was recorded before application of the products and after 2 and 6 hours. Immediately after the 2 hour measurement, the skin on the right arm was cleaned as above, and measured once again. The cream emulsions tested contained glycerine, PCA or urea as humectant.

Measurement of skin water loss (VI)

The increase in skin hydration after application of three emollients (VI) was studied in seven subjects (mean age 36 years) with no clinical signs of skin diseases or dry skin. Using a micropipette, 3 mg/cm² of the emollients was randomly allocated to six areas on the volar forearm. Each product was applied to two areas. Petrolatum without water (Witco), a cream with 27% lipids and 71% water and another cream with 66% lipids and 30% water were tested. Before any measurement, the skin was cleaned with a soft tissue impregnated with dehydrated diethyl ether. Thereafter the basal level of TEWL was measured. After application of the products, the evaporation of water from the surface was measured with the Evaporimeter during a period of 5 or 40 minutes, whereupon the product residue was gently removed by wiping the skin with a soft tissue as above. Immediately after cleaning the skin, the water loss was recorded until the basal level was regained (usually within 15-20 min). Control experiments were performed in order to check whether the released water originated from cream residue on the skin, or from the skin itself. In these experiments the cream containing 71% water was applied to the skin and then immediately removed as described above. In addition, the effect of the cleaning procedure on the water loss was examined by repeated cleaning of the surface.

Determination of the influence of moisturizers on barrier function (VII)

The influence of moisturizers on the skin susceptibility to irritation (VII) was studied in 72 subjects (mean age 37 years) without any skin diseases. The subjects were divided into six groups, each consisting of 8-14 persons. Four groups tested three creams and one gel for 20 days, applying the product twice daily. The other two groups applied two gels and the corresponding placebo three times, with one application in the evening and two on the following day. One cream contained no humectant (A), another

contained 7% glycerine (B) and the third 10% urea (C). The gel used for 20 days contained 10% urea (D). This gel and another gel containing 5% urea (E) were each compared with a placebo gel without urea (F), in a double-blind study. All products were freshly prepared.

Before the products were used, the skin capacitance and TEWL were measured. After completion of the short-term study (three applications) and after 10 and 20 days of treatment, these variables were again measured. In the morning before the measurements the subjects washed their arms. On the second day after completion of the treatments, i.e. the day after capacitance and TEWL measurements, the skin was exposed to aqueous solutions (50 µl) of 10% SLS for 7 h. The following day, the degree of irritation was evaluated by visual scoring and by measurement of TEWL and cutaneous blood flow.

Determination of the influence of lipids on surfactant-irritated skin (VIII)

Twenty one healthy subjects, age 22-57 years, 7 men and 14 women, with no visible signs of skin diseases, participated in the study. In total nine different lipids were applied to normal and surfactant-irritated skin. The parameters assessed were visible signs of irritation and objectively measured TEWL and superficial skin blood flow. The lipids tested were hydrocortisone, petrolatum, fish oil, borage oil, sunflower seed oil, canola oil, shea butter and fractions of unsaponifiable lipids from canola oil and shea butter. Water was included as a control.

The effect of the lipids on normal skin was studied on the volar aspect of the right forearm using a single patch test exposure together with visual and instrumental evaluation of the degree of irritation. Fifty microlitres of each substance was pipetted into large aluminium chambers (12 mm, Finn Chambers, Epitest Oy, Finland). One layer of filter paper was used in the chambers to keep all substances except petrolatum and the hydrocortisone cream in place. The chambers were attached to the skin with adhesive tape (Scanpore, Norgeplaster, Norway). After 48 h of exposure the test substances were removed.

The effect of the lipids on SLS-irritated skin was studied on the left forearm. Prior to application of the lipids, the skin was exposed for 7 h to aqueous solutions (50 µl) of 14% SLS (Ph. Eur.) contained in 10 large aluminium chambers. Upon removal of the patches, the skin was gently rinsed with water and allowed to dry. The test substances were then randomly applied to the SLS-treated areas as above and held in place for 17 h.

After removal of the substances, the left and right arm were gently cleaned with a mild soap solution (ACO Mild Tvål, ACO Hud, Sweden) and dried with soft paper. Twenty-four hours later the areas were examined for irritation.

Ethical considerations

All participants gave their informed consent. The studies were approved by the local Ethics Committee.

Statistics

Paired data were used when possible to avoid the effects of interindividual differences among the participants. The Friedmann test was used to determine whether there were significant differences between the treatments in studies III and VIII. All subjective assessments were evaluated by the Wilcoxon signed rank test (IV, VII, VIII). The latter test and Student's *t* test were used for statistical analyses of differences observed in studies I and V and studies II, III and VI, respectively. Correction for multiplicity was done as described by Holm (1979) to get an overall significance level of <0.05 in study VIII. Linear regression analysis was used to calculate correlation coefficients (II, III). A P level of <0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Characterization of clinically dry skin in atopic patients (I, II)

Topography (I)

For centuries dermatologists have depended on their eyes and fingers to assess changes of the skin. In patients with atopic dermatitis, the skin in non-eczematous areas has a dry-looking appearance and the surface often feels rough to the touch (Linde 1989a). The feeling of roughness reflects a structural abnormality of the skin surface with a change in the surface morphology, as visualized by use of a replica technique and SEM (Linde 1989a). Using profilometric analysis of replicas from dry atopic skin, the topography was quantitated and compared with that of normal skin. The dry surface was really rough (Fig. 2).

In dry atopic skin we found a significant increase in the roughness parameters R_a , R_q , and R_v and a decrease in the number of peaks, indicating higher but

fewer peaks than in normal skin. Both atopic and normal skin exhibited a negative skewness, indicating that the skin relief is mainly formed of plateaus, as may be expected from the visual appearance of the skin topography. No significant difference was found between atopic and normal skin regarding skewness. Also, the values for Δ_a and kurtosis were similar. These results indicate that normal and dry atopic skin have approximately the same profile, which is further substantiated by the finding that the two bearing area curves were almost overlapping.

Friction, capacitance and transdermal water loss (II)

The skin at three locations in patients with atopic dermatitis was further characterized using three non-invasive instruments: a Corneometer, a friction instrument and an Evaporimeter. Data from these measurements were compared with each other and related to the severity of the clinical appearance of dry skin.

The skin in atopic subjects had significantly lower friction than normal controls. Furthermore, the capacitance was lower in atopic subjects, which is in conformity with previous reports (Werner 1986, Thune 1989, Berardesca 1990). In the controls, the skin on the back showed a higher capacitance than that on the forearm. This is in accordance with previously reported data (Cua 1990). We also found higher friction on the back than on the forearm in the control group, which does not support previous findings (Cua 1990).

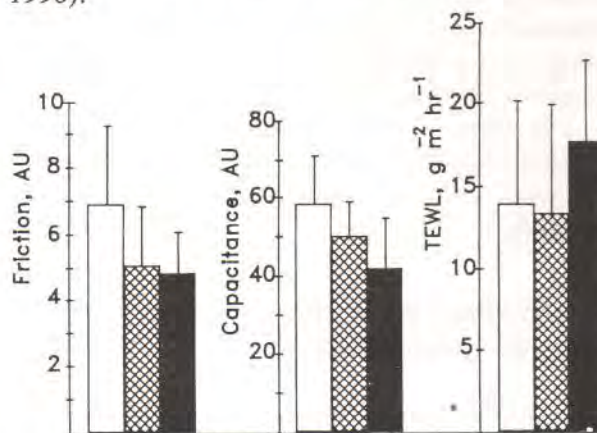


Figure 3. Relationship between biophysical data of the skin and clinical dryness score in atopic skin. The empty columns represent clinically normal skin, the cross-hatched columns rough skin with slight scaling, and the filled columns rough skin with scaling. AU=arbitrary units.

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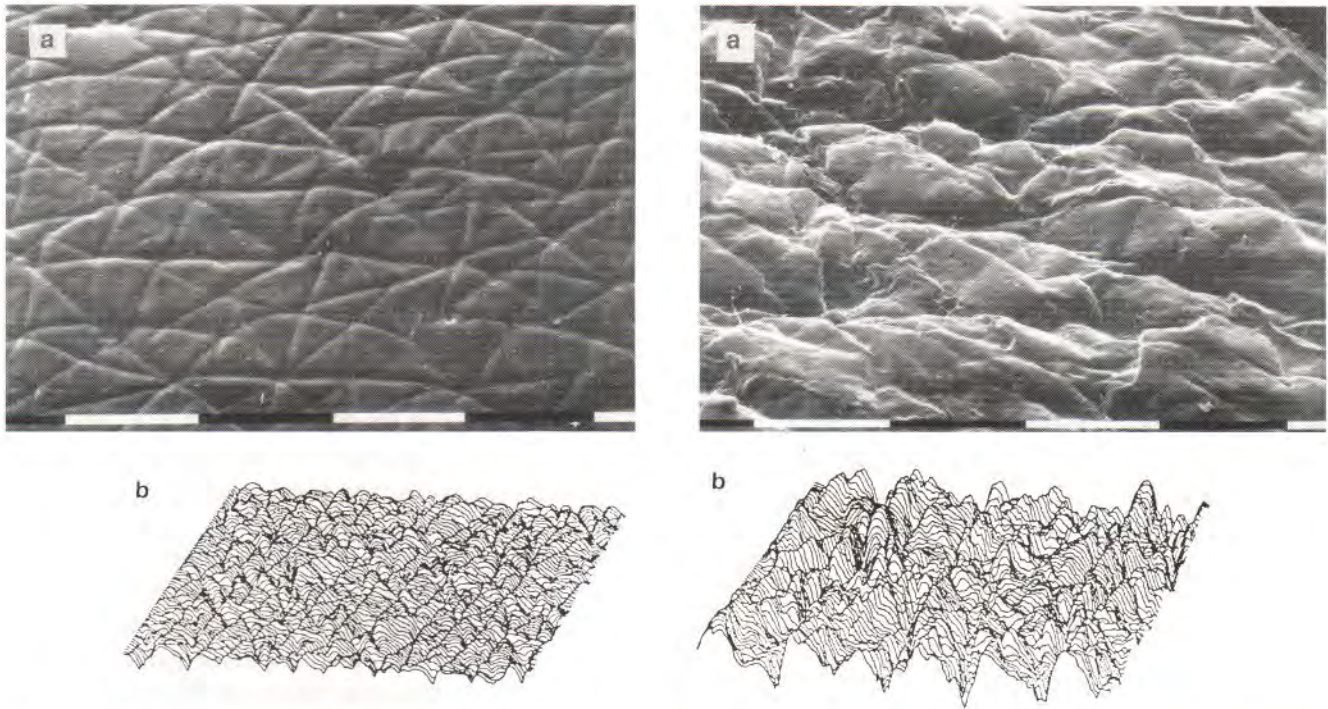


Figure 2. A. Scanning electron micrographs of replicas from normal (left) and dry atopic (right) skin (bar 1 mm). B. Three-dimensional reconstruction of the same replica ($X=x9$, $Y=x9$, $Z=x45$). Note that the major furrows in the micrographs are perpendicular to those in the three dimensional reconstructions.

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The relationship between the clinical appearance of the atopic skin and the instrumental measurements is shown in Figure 3. The correlation between friction and capacitance was significant at most sites, both in the atopic and control groups. The reason for this might be that the presence of loose material on the skin, such as scales, influenced the readings. Scales may act as a dry lubricant in the same manner as graphite and talc and thereby reduce friction. Scales may also reduce the intimacy in the contact between the capacitance probe and the skin, which possibly could give rise to lower capacitance values. Besides scales, the superficial elasticity and roughness of the skin could influence the extent of contact between the probes and the skin. Softer material appears to give a larger contact area, with consequently higher friction, than harder material (*El-Shimi 1977*).

TEWL was significantly increased in all areas in the atopic patients. The present study and recent investigations indicate an inverse relationship between TEWL and skin capacitance (*Berardesca 1990, Thune 1989*). However, in most areas we found no significant correlation between these two parameters. A lack of correlation between TEWL and capacitance was also noted in a recent study on normal skin (*Treffel 1994*). A possible reason for this could be that properties at

different depths of the stratum corneum contribute to the readings with varying intensity. Furthermore, not only dry skin but also hydrated skin has a reduced diffusional resistance to water (*Blank 1984*). Thus, the relationship between TEWL and the water content of the stratum corneum is complex.

Effects of skin care products and some ingredients (III-VIII)

Removal of the outermost skin layer by the use of a scrub cream (III)

Consumers often report increased smoothness of the skin after the use of scrub creams. In the present study we showed that the use of a scrub cream on normal skin removed the outermost layers of the stratum corneum, resulting in measurable changes of the surface features which are compatible with the perception of increased smoothness. We captured and analysed the topography using the replica technique and profilometry. The skin removal was evaluated by exposing the skin to SLS and comparing the degree of

irritation obtained after scrubbing with that occurring after different numbers of tape strippings.

Degree of skin removal

After scrubbing of the skin or stripping it with tape, no changes were observed visually. However, the susceptibility of the skin to SLS increased with increasing numbers of tape strippings, as assessed both visually and with use of an Evaporimeter and a laser Doppler flowmeter. The same order of rank of 0, 3, 6 and 12 tape strippings was obtained with all three methods of evaluation, and the increases in TEWL and blood flow were found to be linearly related to the number of strippings (Fig. 4).

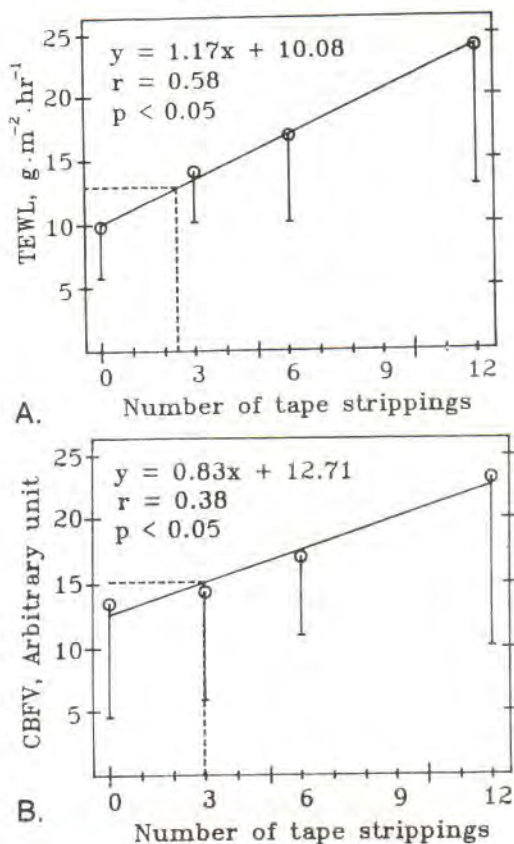


Figure 4. (A) Transepidermal water loss (TEWL) and (B) cutaneous blood flow (CBFV) after challenge with SLS as a function of number of tape strippings. The dotted line shows the result from the scrubbed area. Results are the mean from nine individuals. The bars indicate S.D. Reprinted with permission from J Soc Cosmet Chem (Lodén 1990 (III)).

The effect of scrubbing could then be compared with that of stripping, using these two "standard curves". The results indicate that scrubbing corresponded to about three tape strippings. The amount of material removed by serial stripping of the

skin and during normal desquamation has been investigated by others (Grove 1989, Roberts 1980, Christensen 1978, Kligman 1964, Tregear 1962). From these reported data we estimated roughly that in the present study the scrubbing removed skin material that otherwise would have become lost within the next two to six days through the normal desquamation.

Changes in topography

Removal of the superficial portion of the stratum corneum by scrubbing altered the topographical features of the skin (Table 6). The profilometry analysis showed that the values of the amplitude parameters were decreased by the scrubbing. Another significant change in the topographical data was an increase in the number of peaks (R_n). In our previous study on dry rough skin in atopic patients (I) we found that the rough skin showed an increase in the amplitude parameters and a decreased number of peaks. Thus, it appears that amplitude parameters and R_n can be used to describe perceived differences in the smoothness of the skin. We also noted that R_n decreased with increasing age, which further validates the use of R_n as an indicator of smoothness, since skin smoothness generally is assumed to decrease with age.

From a methodological point of view, we found that an unwashed skin may not yield a faithful replica of the true skin surface, probably due to filling of the irregularities of the surface with endogenous and exogenous substances. There was also a tendency towards higher amplitude parameters after washing of the skin with a detergent. This observation strongly emphasizes the importance of analysing a clean surface if the effects of cosmetics on the skin topography are to be examined.

Changes in the frictional response following application of moisturizers (IV)

Although moisturizers are all oil-in-water emulsions, they differ in their sensory properties. In a consumer study, such differences were found to be of importance with regard to the acceptance of the products. The differences in liking could be attributed to the frictional behaviour of the products. The subjectively perceived properties of five moisturizers and their relationship to objectively measured friction were investigated using a newly developed friction instrument and panellists trained in sensory evaluation. The viscosity of the tested moisturizers ranged from 5,000 mPas to 45,000 mPas and increased from cream A to cream E.

Table 6. Changes in the skin topography after using a scrub cream.
Mean values from 11 individuals \pm S.D.

Roughness parameter	Before scrub	After scrub	Significance
R_a (μm)	18.0 ± 2.8	15.9 ± 2.1	$P < 0.05$
R_q (μm)	22.6 ± 3.4	20.0 ± 2.5	$P < 0.05$
R_y (μm)	119.8 ± 13.9	108.7 ± 11.0	$P < 0.05$
R_n (number of peaks/cm)	27.0 ± 3.7	31.3 ± 5.2	$P < 0.05$
λ_a (wavelength, μm)	141.4 ± 16.2	129.8 ± 18.3	$P < 0.05$
Δ_a (radians)	0.80 ± 0.13	0.77 ± 0.11	not significant
R_{sk}	-0.34 ± 0.12	-0.37 ± 0.12	not significant
R_k	3.06 ± 0.27	3.13 ± 0.27	not significant

Instrumental and sensory evaluation of friction

During spreading of the moisturizers A-E, the trained panellists rated the friction of the skin to be lower than that of untreated skin, a result that was also obtained with the friction instrument. The sensory evaluation and the instrument showed that the cream with the highest viscosity (E) gave significantly higher frictional resistance during application than a cream with lower viscosity (B) (Fig. 5). The panellists also ranked the creams in the same order as could be expected from their viscosity, indicating that during application the frictional resistance depends on the viscosity of the product. Similar results have been reported after application of silicone oils to the skin (El-Shimi 1977). In a separate consumer test, the cream with the highest viscosity (E) was considered to be too stiff and the subjects preferred the application of the cream with lower viscosity (B).

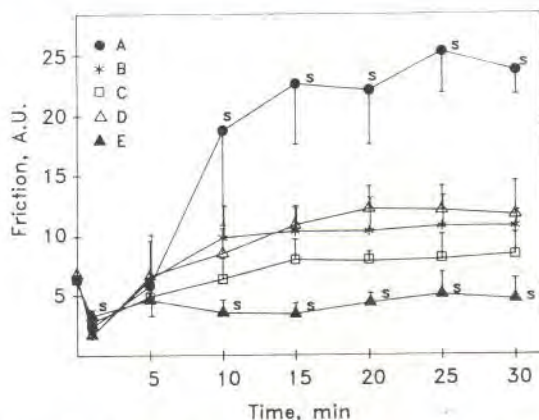


Figure 5. Instrumental evaluation of the frictional resistance after application of five moisturizing creams A-E. The results are mean values from seven subjects. The bars indicate S.D. A.U.=arbitrary units. s=significantly different from cream B. Reprinted with permission from J Soc Cosmet Chem (Lodén 1992 (IV)).

Since water and other volatile agents evaporate, there was a rise in friction after application of the products, except for the cream with the highest viscosity (Fig. 5). The area treated with that cream gave about the same resistance as the untreated skin. The time course of the change in friction was almost the same with the two types of friction measurements. However, the panellists tended to consider that the friction decreased after 5 minutes, whereas the instrumental measurements indicated higher and more persistent friction.

Acceptance testing

The consumers rated the application of cream B, with intermediate viscosity (7,000 mPas) to be more pleasant than that of cream E with the highest viscosity (45,000 mPas) (Fig. 6). Cream A (5,000 mPas) was considered to be more slippery than product B on application. Fifteen minutes after application of the creams, the consumers rated the skin area treated with cream B to be significantly more pleasant than the area treated with product E and considered this to be due to the slippery feeling of the latter area. This is probably due to the higher concentration of oils in cream E than in the other tested products. A large amount of non-absorbed oil residue on the surface allows the friction probe and the fingers to ride freely on the oil film. These findings support earlier observations concerning greasiness and skin friction (Nacht 1981). The more greasy and unpleasant the products were perceived as being, the lower the skin friction that was obtained (Nacht 1981).

Studies of the correlation between objective observations made with instruments and evaluations by panellists are important in order to determine which part of the sensory evaluation can be most satisfactorily accomplished by the use of an instrumental approach. This study shows that the

friction meter gives readily quantifiable data on the frictional response following application of creams. The results were comparable with those obtained by sensory evaluation. Hence, the study indicates that the friction instrument can be used to predict the degree of liking of moisturizers during application and shortly afterwards.

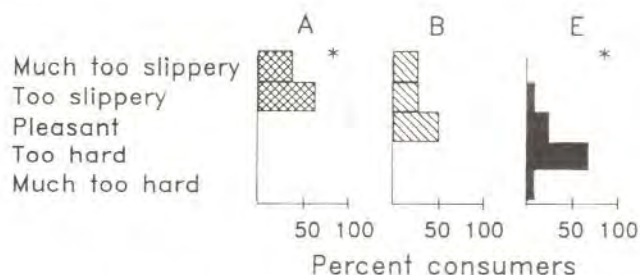


Figure 6. Sensation of the skin feel during application of the creams A, B, and E. The distribution is based on results from 11 individuals. Asterisks denote significantly differences from the results for cream B. Reprinted with permission from J Soc Cosmet Chem (Lodén 1992 (IV)).

Increase in skin hydration after single application of moisturizers (V, VI)

One primary goal with the use of moisturizers is to increase the skin hydration. A variety of techniques for assessing such effects have been used, but each one suffers from limitations and pitfalls (Lodén 1994, Potts 1986, Lévêque 1983ab, Serup 1992a). In the present study we found that measurements with a Corneometer were difficult to interpret (V), whereas an Evaporimeter could be used to selectively measure the build-up of water in the skin due to a single application of a moisturizer (VI).

Capacitance measurements (V)

Each product was applied to two areas on the skin. Two hours after application, the capacitance was significantly increased on all treated sites. Cleaning of one of the areas caused a prompt and significant decrease in the capacitance value on the areas treated with the creams, whereas a tendency towards a higher value was noted on the petrolatum-treated sites. These findings indicate that product residues on the skin surface influence the capacitance. The emulsions contained different humectants, e.g. glycerine, PCA and urea. The presence of these substances on the skin surface may well have influenced the measurements, both in themselves and by attracting water to the surface. In a study by Wepierre (1977), lipid material

that simply remained on the surface was found to depress the electrical response, so that the moisturizing effects were underestimated.

Measurement of skin water loss (VI)

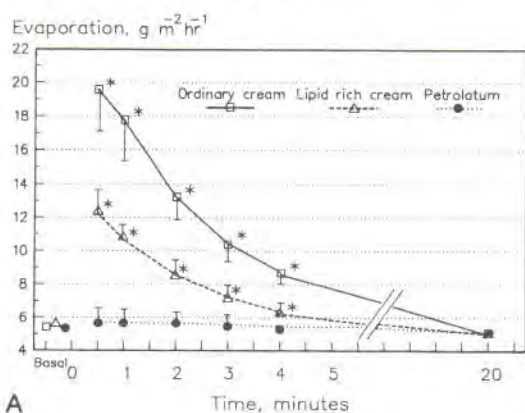
To circumvent the limitations of capacitance measurements for detecting increases in skin hydration, we designed a method whereby an Evaporimeter could be used to study the hydration after application of emollients (VI). In this study, water-containing moisturizers were found to induce an immediate increase in hydration by delivering of some of their water to the skin. The increase appeared to be related to the amount of water in the products. Similarly, a relationship between degree of occlusion and skin hydration was demonstrated. The build-up of water in the skin was detected by removal of product residue, which resulted in an increased water loss from the surface.

To clean the skin, soft paper impregnated with dehydrated diethyl ether was used. A possible drawback of this cleaning procedure is that it may cause a temporary change in the water barrier function, or increase the sweat gland activity. However, control experiments on untreated skin showed that the cleaning procedure did not influence the water loss. Furthermore, in control experiments it was found that the cleaning efficiently removed cream residues, eliminating the possibility that the detected water loss was due to such residues on the skin surface. In previous desorption experiments, the skin was cleaned solely by wiping the surface with soft paper, or not cleaned at all (Thune 1989, Berardesca 1990, Tagami 1982, 1985).

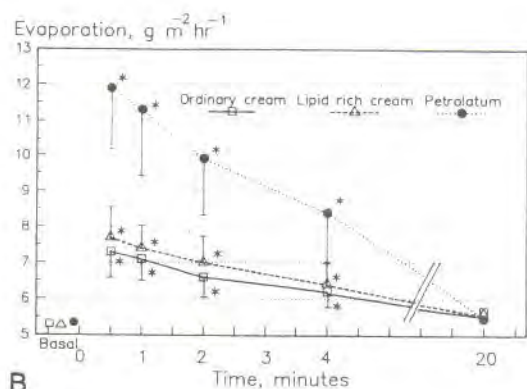
The tested products with different contents of water were applied to the skin and allowed to remain on the surface for 5 minutes. They were then removed by cleaning of the surface. Hydration achieved from the products resulted in an increased water loss immediately after the cleaning, which was due to release of excess water in the skin (Fig. 7A). The emulsion containing most water (71%) gave the highest increase in water loss after cleaning, followed by the one containing 30% water. After removal of petrolatum with no water, there was no increase in water loss. The release of absorbed water declined with time, and the basal TEWL was regained within 20 minutes.

Using the same technique, we found that when there was a reduction in TEWL, due to occlusion, there was a corresponding increase in skin hydration. The products were applied to the skin and the change in water loss was recorded. The water loss from the emulsion-treated surfaces was significantly increased

during the first minutes after application, whereas the water loss from the petrolatum-treated area was reduced (-57%). It is known that emulsions lose most of their water within 15 minutes after application (Blichmann 1989, Wepierre 1982, Rietschel 1978). Forty minutes after application of the products, the water loss from all treated sites was significantly lower than the pre-application value. As expected, petrolatum was the most occlusive product among those tested, and reduced TEWL by about 45%, whereas the others reduced this loss by about 16%. This decrease in water loss indicates an increased moisture content in the skin. After removal of the product residues, this increase in moisture could easily be detected by using the Evaporimeter to monitor the release of trapped water (Fig. 7B). The increase in water loss was temporary and after about 20 minutes the basal TEWL was regained.



A



B

Figure 7. Evaporation of water from the skin after removal of product residue.

The skin was exposed to the products for 5 (A) and 40 (B) minutes.

The results are mean values from seven subjects. The bars indicate S.E.M. and the asterisks denote results significantly different from the basal level. Reprinted with permission from Acta Derm Venereol (Stockh) (Lodén 1992 (VI)).

The amount of water that is released from the skin is represented by the area under the evaporation curves. Thus, the increase in the amount of water in the skin can be estimated roughly from these curves. The results indicate that the largest increase is obtained after absorption of water into the skin. However, it must be borne in mind that this increase is only temporary, in contrast to the more long-lasting effects of occlusion. Absorption of product water into the skin was unexpected, but this finding has recently been verified (Querleux 1994).

The increase in skin hydration due to application of water-containing moisturizers may have clinical relevance in potentiating the effects of previously applied drugs. In a recent study on eczematous skin it was found that hydrocortisone was stored in the skin, and that application of a moisturizer released the corticosteroid from the reservoir (Turpeinen 1991). The content of propylene glycol in the moisturizer was thought to be responsible for this release (Turpeinen 1991), but it might well also have been due to the increased hydration.

Changes in barrier properties after repeated application of moisturizers (VII)

Few studies have addressed the possible impact of moisturizers on normal skin. Concern has been expressed that urea-containing moisturizers might impair the chemical barrier function of skin against toxic chemicals (Hellgren 1974), due to the keratolytic, hydrating and permeability increasing properties attributed to urea (Hellgren 1974, Swanbeck 1968, Wohlrab 1984, 1989, 1990, Allenby 1969, Kim 1993, Beastall 1986). In the present study urea-containing moisturizers were proved to influence both TEWL and the apparent susceptibility to SLS-induced irritation. Three applications of a gel containing 5% urea increased TEWL, whereas treatment with 10% urea for 10 and 20 days decreased TEWL. Increased capacitance was found after three applications of 10% urea, and the increase persisted after treatment for 10 days, but not after treatment for 20 days. Besides these changes, the urea-containing products also reduced the irritative response to SLS, the reduction being evident both on visual evaluation and from measurements of TEWL and cutaneous blood flow (Fig. 8). This effect was observed after only three applications and remained after treatment for 20 days. The other tested moisturizers increased the skin capacitance, but induced no changes in TEWL or reactivity to SLS.

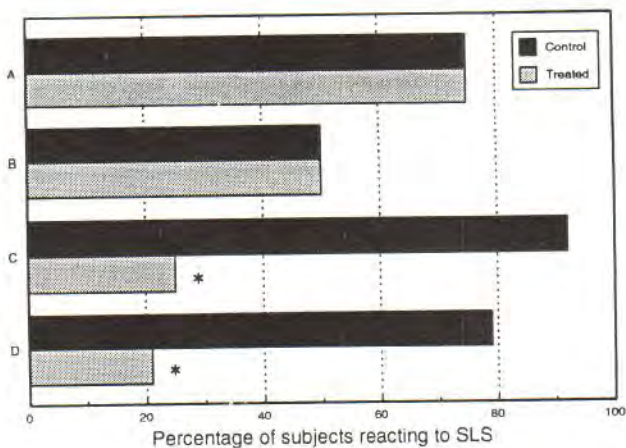


Figure 8. Irritative response to sodium lauryl sulphate (SLS) after treatment with the moisturizers A-D for 20 days, evaluated as the percentage of skin areas showing visible signs of irritation. Products A and B contain no urea, and C and D contain 10% urea. Each cream was tested on 8-14 individuals. Asterisks denote results significantly different from the control.

The increase in TEWL after three applications of a gel containing 5% urea might be due to an increase in skin hydration, as indicated by the increased skin capacitance. It is also possible that urea may have an immediate effect on the barrier lipids, since urea is a hydrophobic bond breaker and may break up the micellar structure (Davis 1971). Modifications of the barrier lipids may explain the penetration enhancement effect of urea observed in previous experiments (Wohlrab 1984, 1989, 1990, Kim 1993, Beastall 1986). However, the decrease in TEWL and the lower irritative response to SLS after long-term treatment with 10% urea were unexpected. Besides other effects on the barrier lipid structure, an increase in corneocyte size has to be considered. Larger corneocytes are reported to decrease the skin permeability (Rougier 1988, Denda 1994, Potts 1991) and long-term treatment with urea has been reported to reduce epidermal cell proliferation (Wohlrab 1975, Wohlrab 1976). However, the question as to whether urea can influence the size of the corneocytes in normal skin has not been addressed experimentally.

The mechanism responsible for the decreased susceptibility to SLS after treatment with urea is not known. Since the change in susceptibility appeared after only a few applications, it seems unlikely that urea altered the morphology of the stratum corneum. Another possible explanation could be trapping of SLS in the stratum corneum. Urea might disperse keratin and expose previously unavailable water-binding sites (Swanbeck 1968), which in turn might possibly retard certain substances in the stratum corneum (Stüttgen

1989). Recent findings also indicate that SLS might form a reservoir in the skin, since the penetration of SLS continues after removal of an SLS-containing patch from the skin surface (Fullerton 1994).

Reduction in surfactant-induced irritation by the application of lipids (VIII)

In the present study we found that canola oil and its sterol-enriched fraction reduced SLS-induced irritation. On normal skin, neither the tested lipids nor the hydrocortisone cream induced any significant irritation, as assessed visually and with the instruments. When the products were applied to surfactant-irritated skin, significant differences were observed. Treatment with hydrocortisone resulted in a significant reduction of the degree of irritation compared with than water. The anti-inflammatory activity of hydrocortisone is well known (Kaidbey 1974). Furthermore, application of canola oil and its sterol-enriched fraction caused a significant reduction of TEWL compared with treatment with water (Fig. 9). Canola oil has a relatively high content of sterols, mainly β -sitosterol, campesterol and brassicasterol in descending order (Homberg 1985). None of the other lipids had any significant effect on the degree of irritation. Neither fish oil (rich in eicosapentaenoic acid) nor borage oil (rich in GLA and linoleic acid) influenced the irritation, although they have been linked to anti-inflammatory effects in other studies (Orengo 1992, Danno 1993, Tolleson 1993ab, Miller 1991). Sunflower seed oil (rich in linoleic acid) was found to reduce the abnormally high rates of TEWL in one study of surfactant-irritated rat skin (Prottey 1976), but in another no effect was observed (Blanken 1989).

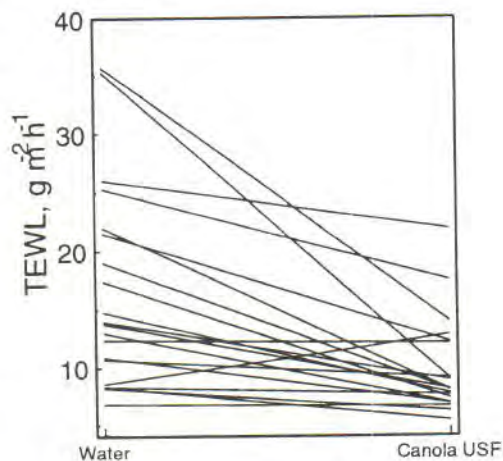


Figure 9. Transepidermal water loss in 21 individual subjects after a single exposure of SLS-irritated skin to water and the sterol-enriched fraction of canola oil for 17 hours. USF=unsaponifiable lipids.

Our findings that canola oil and its sterol-enriched fraction ameliorated the irritation, emphasize that lipids used in moisturizers not only form an inert, epicutaneous, occlusive membrane, but may penetrate and influence the barrier properties of the skin. Further studies are needed to elucidate the mechanism and the time course of the effect.

GENERAL DISCUSSION

Measuring methods

During recent years a number of highly developed non-invasive methods for evaluation physiology and pathology of the skin have been brought into use. These techniques are important research tools in investigating the properties of the skin and in determining therapeutic effects. In the present investigation, we introduced a newly developed friction instrument for evaluation of the frictional response of normal skin after application of moisturizers, and of dry skin (II, IV). The other instruments used in our studies are commercially available and widely applied, thus providing opportunities for interlaboratory comparisons. Furthermore, internationally agreed guidelines for measurements of TEWL and cutaneous blood flow have been published, which facilitates correct handling of the instruments and interpretations of the results (*Pinnagoda 1990, Bircher 1994*). It is to be hoped that similar guidelines will follow for other methods. It is important to confirm that the instrumental readings correspond to perceptible or otherwise meaningful properties of the skin.

The friction instrument has been validated in a number of studies (*Olsson 1994*). Our validation included parallel assessments of the skin friction after application of moisturizers, by use of the instrument and by individuals trained in sensory analysis (IV). The two types of measurement gave comparable results, indicating that the instrument can be used to measure the frictional resistance of the skin (IV).

The friction instrument is easy to handle and appears to give a true reading of the skin friction. Another technique which provides valuable information about the tactile properties of the skin is analysis of the skin topography (II, III). However, this is rather time consuming. Other easily performed measurements are those of skin capacitance, blood flow and TEWL. The Corneometer for skin capacitance measurements is by far the most easily handled instrument among those used in the present studies. However, although it is claimed to be valid for

measurement of skin hydration, these results are difficult to interpret (II, V, VII).

TEWL serves as an important and sensitive indicator of the integrity of the stratum corneum. In order to further increase our understanding of the skin permeability, we developed a method whereby the skin response to an irritative stimulus could be used as an indicator of the barrier function. This concept involved a closed patch exposure to rather high concentrations (8 and 10%) of SLS for 7 h, and this proved useful for detecting changes in percutaneous absorption (III, VII).

Since non-invasive measurements can be performed repeatedly on the same skin area, the changes in TEWL and skin capacitance due to long-term use of moisturizers could be followed (VII). Likewise, in the evaluation of the use of a scrub cream on the skin topography, we found that three replicas from the same skin area could be used to demonstrate the effects both of ordinary cleaning and of scrubbing (III). The results indicated that uncleaned skin gave smoother replicas of the surface than the washed skin, and that the use of a scrub cream made the skin less rough (III). Thus, fillings in the valleys may prevent a true replica of the surface from being captured (III).

Dry skin and moisturizers

The surface topography, friction, capacitance and TEWL were measured in order further to characterize one state of clinically dry skin, namely atopic dry skin (I, II). Already in the mid-1980s, reports on the lower water content (*Werner 1986*) and the defective barrier function (*Werner 1985*) in dry atopic skin appeared. We confirmed these findings (II), and also demonstrated the relationship between skin capacitance, friction and TEWL. Unexpectedly, skin capacitance and TEWL were found not to be related to each other. Neither did skin friction correlate to TEWL, but a significant correlation between friction and capacitance was observed (II). The roughness parameters in dry atopic skin were also significantly increased (I). These parameters may well be used to monitor treatment effects on atopic skin.

Dry skin can be treated with moisturizers and emollients. Usually these diminish clinical appearance of dryness, although the exact mechanism underlying the improvements is unknown (*Siskin 1993, Fredriksson 1975, Grice 1973, Dahl 1983, Rosten 1970, GPR 1973, Pope 1972, Frithz 1983, Vilaplana 1992, Middleton 1978, Halkier-Sørensen 1993, Swanbeck 1968, Dunlap 1984, Rattner 1943, Serup 1992a*). Nor has the substance(s) responsible for the effects been identified. Lipids in moisturizers have

long been believed to exert their effects on the skin solely by forming an inert, epicutaneous, occlusive membrane. They are therefore incorporated into formulations on the basis of their technical and sensory properties rather than on their possible impact on the epidermis. That the sensory properties of creams is important in product acceptance was also demonstrated in study IV. The observed differences in acceptance could be attributed to tactile changes in the frictional properties of the skin following product application, as measured with the new friction instrument and assessed by panellists trained in sensory analysis (IV).

Besides changing the frictional response of the skin, moisturizers increase the degree of skin hydration with different rapidity and by different mechanisms (VI). For instance, it was conclusively shown that moisturizers provide water directly to the skin from their water phase (VI). In addition, moisturizers may alter TEWL and the susceptibility to irritant stimuli (VII). The lower degree of SLS-induced irritation observed after treatment with urea-containing moisturizers was not anticipated, but may well be of clinical relevance in reducing the severity of contact dermatitis caused by irritant stimuli (VII). Furthermore, we conclude that lipids used in moisturizers do not only form an inert, epicutaneous, occlusive membrane, but that they may penetrate and influence the barrier properties of the skin (VIII). As also noted for hydrocortisone, canola oil and its sterol-enriched fraction were found to alleviate irritation induced by SLS (VIII). The observed effect of canola oil and its sterol-enriched fraction is probably due to their content of sterols, mainly β -sitosterol, campesterol and brassicasterol, in descending order. The epidermal membrane lipids also contain sterols, and the disruption of the barrier function is followed by an increased synthesis of unsaponifiable lipids (Grubauer 1987, Menon 1985, Feingold 1990), fatty acids (Grubauer 1987) and sphingolipids (Holleran 1991). Application of certain lipids may thus assist the skin in supplying the SLS-damaged barrier with adequate lipids. More detailed studies are needed to elucidate the time course and the mechanism of the impact of topically applied lipids on the skin function. Our findings indicate that moisturizers may have greater impact on the skin than is generally believed. The term "cosmeceuticals", as proposed by Kligman (1993), may be relevant to describe products which contain no recognized medicaments, but nonetheless have medical value.

Future development

While the impression of dry skin is a common disorder, a lack of water may be too simple an explanation for all types of problems covered by the term dry skin, such as redness, scaling, roughness, itching and a feeling of discomfort. It is possible that some conditions need replenishment of other substances than water. Moreover, it would seem reasonable to believe that the true dry skin condition should be treated according to the underlying pathogenesis. For example, atopic dry skin, winter xerotic dry skin, and surfactant-induced dryness may need moisturizers with different ingredients. The fact that the long-term preference for moisturizers varies between individuals supports this assumption. Thus, the acceptance of and compliance with the treatment may not be related solely to the immediate sensory perception of the product, such as odour and tactile characteristics during application (IV), but also to the type of abnormality in the epidermis induced by a number of biological pathways. Changes in the epidermal composition, caused by environmental, dietary, behavioural or genetic factors, or sex-dependent changes may need particular substances to restore the normal homeostasis. For instance, in rough and scaly skin the content of amino acids is changed (Denda 1992, Horii 1989). Furthermore, the lipid composition is altered in several dry skin conditions (e.g. Fulmer 1986, Denda 1992, 1994, Melnik 1988, 1990, Linde 1989b, Imokawa 1991a, Hollmann 1991). The findings that the lipid distribution in normal skin also varies from one anatomical region to another (Lampe 1983) and appears to be sex- and age-dependent (Denda 1993, Imokawa 1991a) may explain why certain skin sites and individuals are more prone to drying out effects than others. Further knowledge is therefore required to gain better insight into the nature of the pathogenesis to which environmental as well as individual factors contribute.

It is obvious that an ideal skin care product should not contain any ingredients that have a harmful effect on the skin, but rather those that correct or prevent an abnormal composition. For instance, urea might be an interesting substance in that it reduces the skin susceptibility to surfactants (VII). Furthermore, the similarity of several skin cream ingredients to components of the intercellular lipids suggests that many of them may have little difficulty in becoming incorporated into such bilayer structures, and thereby affecting the structure and/or the function of the skin barrier (VIII). There is no doubt that topically applied

humectants and lipids are absorbed into the skin (*Batt 1986, Wellner 1993, Lodén 1994, Moloney 1988, Wertz 1990, Dewsbury 1989, Man 1993, Rawlings 1994, Escobar 1992, Tolleson 1993ab, Ghadially 1992*). Furthermore, substances may be converted by enzymes in the skin during their penetration (*Rawlings 1994, Moloney 1988, Lodén 1985, 1990, Wertz 1990*). For instance, esters have been found to enter into metabolism and modify endogenous epidermal lipids (*Wertz 1990, Rawlings 1994*). Esters, fatty acids, cholesterol and NMF have long been used in moisturizers, but research into their benefits to barrier properties is sparse.

A rational strategy for the treatment of the clinical signs and the uncomfortable feeling of dry skin of various origins, would possibly be to use creams that deliver substances that supplement those naturally found in the skin. The promising finding that canola oil and its sterol-enriched fraction improved surfactant-irritated skin needs to be further explored (VIII). The improved understanding of the interactions between topically applied substances and the epidermal biochemistry will inevitably alter the formulation of skin care products from art to science.

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