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NONINVASIVE MEASURING METHODS FOR THE INVESTIGATION OF IRRITANT PATCH TEST REACTIONS

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NONINVASIVE MEASURING METHODS FOR THE INVESTIGATION OF IRRITANT PATCH TEST REACTIONS

A study of patients with hand eczema, atopic dermatitis and controls

Tove Agner

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II. Agner T, Serup J, Handlos V, Batsberg W. Different skin irritation abilities of different qualities of sodium lauryl sulphate. Contact Dermatitis 1989; 21: 184-188.

III. Agner T, Serup J. Individual and instrumental variations in irritant patch-test reactions - clinical evaluation and quantification by bioengineering methods. Clinical and Experimental Dermatology 1990; 15: 29-33.

IV. Agner T, Serup J. Sodium lauryl sulphate for irritant patch testing – A dose- response study using bioengineering methods for determination of skin irritation. Journal of Investigative Dermatology 1990; 95: 543-547.

V. Agner T, Serup J. Seasonal variation of skin resistance to irritants. British Journal of Dermatology 1989; 121: 323-328.

VI. Agner T, Damm P, Skouby SO. Menstrual cycle and skin reactivity. Journal of the American Academy of Dermatology 1991; 24: 566-570.

VII. Agner T. Basal transepidermal water loss, skin thickness, skin blood flow and skin colour in relation to sodium-lauryl-sulphate-induced irritation in normal skin. Contact Dermatitis 1991; 25: 108-114.

VIII. Agner T. Skin susceptibility in uninvolved skin of hand eczema patients and healthy controls. British Journal of Dermatology 1991; 125: 140-146.

IX. Agner T. Susceptibility of atopic dermatitis patients to irritant dermatitis caused by sodium lauryl sulphate. Acta Dermato-Venereologica (Stockh) 1991; 71: 296-300.

In the text these papers will be referred to by their respective Roman numerals.

PREFACE

The studies on which this review is based were carried out during my research fellowship at the Department of Dermatology, Rigshospitalet, Copenhagen, initially under the leadership of the late Gustav Asboe-Hansen, later by Else Svejgaard and by Gunhild Lange Vejlsgaard. I am greatly indebted to all of them for creating the working conditions making this study possible.

As my supervisor and colleague Jørgen Serup has been invaluable. With never ending enthusiasm he has always been ready with guidance and support. His industrious, scientific minded and generous personality has been an inspiration to me.

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Finally I want to thank my husband Peter Damm for skilful advice concerning scientific methods and for love and support during my work, and my children Julie and Emilie for kindly distracting and broadening my mind.

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Tove Agner *Copenhagen, February 1992*

AIMS

To

1. characterize and validate a standard test for skin susceptibility, as a basis for further studies.
2. evaluate the use of selected noninvasive measuring methods for examination of unaffected skin and for quantification of irritant skin responses following standard testing.
3. study the susceptibility of clinically normal skin to standard irritant trauma under varying physiological and pathophysiological conditions.

ABBREVIATIONS

TEWL = transepidermal water loss
SLS = sodium lauryl sulphate
DMSO = dimethyl sulfoxide
RH = relative humidity
MED = minimal erythema dose

CONTENTS

INTRODUCTION

In a recent epidemiological study, irritant contact dermatitis constituted 35% of hand eczema cases as compared to 19% for allergic contact dermatitis and 22% for atopic hand eczema *(Meding & Swanbeck 1989)*. The diagnosis of irritant contact dermatitis was based on exposure to irritant substances related in time to periods of eczema and on the exclusion of other diseases. Irritant contact dermatitis may also sometimes complicate other types of dermatitis *(Andersen et al 1987)*.

The pathogenesis of irritant contact dermatitis is generally accepted to be multifactorial *(Malten 1981)*. Development of irritant contact dermatitis depends on: 1) the character and intensity of the chemical/physical trauma, 2) exposure time or interval between repetition of a given trauma, 3) current susceptibility of the skin. Exposed to the same exogenous conditions, some subjects will develop eczema, while the skin of other subjects will remain intact. Knowledge of endogenous as well as exogenous variables which influence skin susceptibility is valuable in the prevention of irritant contact dermatitis. Identification of high-risk subjects, followed by information, counselling and surveillance may prevent the development of (hand)-eczema, which may often become chronic and sometimes perhaps permanent *(Wall & Gebauer 1991)*.

SKIN TESTS

Many skin tests have been proposed for the purpose of identifying individuals with sensitive skin. A thorough review is given by *Frosch (1985, p 8-9)*.

The *alkali resistance test (Burckhardt 1947)* evaluates the resistance of the stratum corneum. Increased sensitivity to alkali was reported in eczema patients, and the test was proposed as a tool for pre-employment testing *(Burckhardt 1964)*. *Bjørnberg (1968, p 119)* was, however, unable to confirm the usefulness of the test, and due to inconsistency in its results the test has now generally been abandoned. Recently, however, *Schulz & Korting (1987)* claimed simultaneous determination of alkali resistance on the back of the hand and on the thigh to be useful in the recognition of an eczematous disposition, though no statistical evidence was presented.

The *ammonium blistering time*, determined as the minimal blistering time after exposure of the skin to ammonia, was proposed as a variation of the alkali test. The time required to form a blister reflects the number of cell layers in the stratum corneum *(Frosch & Kligman 1977)*. In the *DMSO test* the intensity of weal response after exposure to DMSO (dimethyl sulfoxide) is graded, and is reported to reflect the permeability of the skin barrier *(Frosch et al 1980)*. Because of its convenience the DMSO-test was proposed as a suitable diagnostic tool for the diagnosis of sensitive skin. Sensitivity to *UV-light* has also been proposed as identifying subjects with delicate skin *(Frosch & Wissing 1982)*.

Patch testing has been widely used in the study of skin irritation from a wide range of chemicals (Table 1). Repetitive patch testing, such as the 21-day cumulative test *(Lanman et al 1968)*, the soap chamber test *(Frosch & Kligman 1979a)*, the chamber-scarification test *(Frosch & Kligman 1976)* and repeated open application test *(Hannuksela & Salo 1986, Lammintausta et al 1988)* was designed primarily for the evaluation of toxic effects of different chemical substances.

In recent years, 24 h patch testing with sodium lauryl sulphate (SLS) has been a frequently used tool in experimental studies of irritant skin reactions (Table 2).

Standardization of irritant patch testing

Variation. Knowledge of variation in skin responses to identical patch tests is essential to the design of experimental studies. Estimation of sample size can then depend

Table 1. *Some primary irritants used for patch testing*

Irritant	Reference
Organic solvents	
Kerosene	Frosch & Wissing 1982
Propylene glycol	Warshaw & Hermann 1952 Björnberg 1968 Hannuksela et al 1975 Wahlberg & Nilsson 1984 Willis et al 1989
Detergents	
Benzalkonium chloride	Björnberg 1968 Holst & Möller 1975 Wahlberg et al 1985 Agner & Serup 1987 Willis et al 1988b
Sodium lauryl sulphate	see Table II
Acids and alkalis	
Hydrochloric acid	Björnberg 1968 Agner & Serup 1989 (I)
Sapo kalinus	Bjørnberg 1968 Holst & Möller 1975 Agner & Serup 1987
Miscellaneous	
Cantharidin	Björnberg 1968
Croton oil	Björnberg 1968 Johnson et al 1971 Coenraads et al 1975 Frosch & Wissing 1982 Agner & Serup 1987 Willis et al 1988b
Dithranol	Mustkallio 1979 Willis et al 1988b
Mercury bichloride	Björnberg 1968
Nonanoic acid	Wahlberg & Maibach 1980 Willis et al 1988b Agner & Serup 1989 (I)
Phenol	Blanken et al 1986
Thymoquinone	Coenraads et al 1975

Table 2. *Different concentrations of SLS used for occlusive patch testing for 24 h*

Patch test	Anatomical region	Concentration	Reference
Finn Chamber 12mm	forearm	0.50-2.00%	Blanken et al 1986
Silver patch	back	0.25-2.00%	Bruynzeel et al 1982
van der Bend	back	4.00%	Coenraads et al 1975
Al-test	forearm	10.00%	Dahl & Trancik 1977
Finn Chamber 12mm	forearm	0.25-1.00%	de Boer et al 1990
Finn Chamber 20mm	arm/back	2.00%	Freeman & Maibach 1988
Duhring chamber	arm/back	0.25-2.50%	Frosch & Wissing 1982
Finn Chamber 12mm	scapula	2.00%	Goh & Chia 1988
Al-test	upper arm	0.50%	Holst & Möller 1975
Finn Chamber 8mm	back	0.50-1.00%	Lammintausta et al 1987a
Finn Chamber 12mm	upper arm	1.00%-10.00%	Serup & Staberg 1987
Plastic chamber (Agfa Bayer, 22mm)	forearm	0.10-0.50%	Tupker et al 1990b
Finn Chamber 12mm	forearm	2.00%	van der Valk et al 1984
Al-test	back	0.10-2.00%	Wahlberg et al 1985
Plastic chamber (Hilltop lab.)	forearm	0.12-3.00%	Wilhelm et al 1989
Finn Chamber 8mm	forearm	1.00-5.00%	Willis et al 1988b*

* 48 h application

on statistical calculations based on previous investigations using the same methods. Variation in skin responses within the same individual to identical irritant patch tests has been claimed to be considerable *(Freeman & Maibach 1988)*. *Dahl et al (1984)* found that, for simultaneous patch testing with SLS, corresponding sites on the right and the left side were scored identically in only 53% of cases. In that study, however, Al-test patches, less adequate for irritant patch testing *(Frosch & Kligman 1979b)*, were used. Using large Finn Chambers (12 mm) we found that 84 % of SLS patches were visually scored identically when tested simultaneously on right and left arms *(Agner & Serup 1990 (III))*. Some of the variation in patch test responses may be related to the traditionally subjective reading of the reactions, and may be overcome by the use of more objective methods *(Wahlberg 1988)*. However, to keep variation to a minimum, standardization of the patch test procedure is essential.

Standardization of the patch test procedure. *Magnusson & Hersle (1965a)* found that the quantity of test solution per mm^2 skin was of importance for the skin response. *Frosch and Kligman (1979b)* demonstrated that larger quantities of test solution gave more intense skin reactions, though concentration of the irritant was kept the same. Comparing Finn Chambers (diameter 8mm) with Duhring chambers (12mm), more intense reactions with the latter were noticed, these being larger as well as deeper than the Finn Chambers. In accordance with these observations, the Duhring chamber, the 12 mm Finn Chamber or even larger chambers may be more suitable for irritant studies, since smaller test areas may be too limited to elicit an irritant response.

When substances in aqueous solution are tested, the interval from wetting of the patch until application should be standardized. The evaporation rate of aqueous solutions from Finn Chambers was reported as 1 mg/3 min *(Fischer & Maibach 1984)*. It has been demonstrated that evaporation from the patch test before application inhibits the inflammatory response, even though the relative concentration of the irritant is increased by the process *(Dahl & Roering 1984)*. Exposure time should be graded according to the irritant potency of the test substance applied *(Novak & Francom 1984)*. The time of evaluation of the skin response is important since some irritants may cause delayed reactions. When noninvasive measurements of the skin response are made, the interval between removal of the patch and the measurements should allow for a period of increased evaporation following occlusion. For measurement of transepidermal water loss, this period was previously estimated as 3 h *(Baker & Kligman 1967)*, but in the set-up used throughout the present studies we found steady evaporation values after 1 h *(Agner & Serup, submitted)*. The interval should be chosen in accordance with the measuring device used, since the time for the most accurate assessment of the skin response may also vary according to these parameters *(Agner & Serup 1990 (IV))*.

Release of test substance. Another source of variation may be the release of test substance from the chamber/filter paper disc to the skin. This issue was addressed in vivo and in vitro, using SLS in aqueous solution and in different

gels: delivery from aqueous solutions was better than from gels, and delivery from the test system was found not to be a major source of variation in irritant patch testing with SLS in aqueous solution *(Agner et al 1990a)*.

Single versus repetitive, open versus closed tests. The occlusive set-up in patch tests will influence the strength and possibly the kinetics of the skin response to the applied substance, and the irritant abilities of the test substance cannot directly be compared to open applications. A single challenge of the skin with an irritant insult is a momentary reflection of skin susceptibility, which does not take into account the cumulative effect of irritation or the repair mechanisms of the skin. Repetitive challenges do allow for these effects. The correlation coefficient between single and 4 day repetitive exposure to patch testing with SLS was found to be 0.63 *(Pinnagoda et al 1989a)*. Utilizing repeated open application of SLS for 5 days as well as a single 24 h patch test with SLS, only the degree of skin damage caused by the repeated open test was found associated with prior skin complaints *(Lammintausta et al 1988)*. The reaction pattern to SLS patch tests also differed from that evoked by open, cumulative SLS irritation *(Lammintausta et al 1987a)*. In the latter 2 studies, however, small Finn Chambers (8 mm), less suitable for irritant patch testing, were used, which may have biased the results.

Primary irritants

A primary irritant is a substance which will damage the skin by direct cytotoxic action *(Kligman & Wooding 1967)*, without preceding sensitization. Some important characteristics for experimentally used primary irritants were proposed by *Wahlberg & Maibach (1980)*: no systemic toxicity, no carcinogenic effect, not a sensitizer, chemically well defined, no extreme pH value and causing no cosmetic inconveniences to exposed subjects. After evaluating a broader spectrum of irritants in preliminary studies, we found that SLS and nonanoic acid fulfilled these criteria.

Qualitative differences in skin response to different irritants. Primary irritants include chemical substances with varying irritant properties, and it may be assumed that the skin response will depend upon the precipitating irritant, reflecting the different ways in which different chemicals may penetrate and interact with components of the skin. This assumption is supported by the different clinical *(Bjørnberg 1968, pp 93-99)* and histological *(Willis et al 1989)* appearance elicited by different irritants, and by differences in skin response assessed by replica technique *(Agner & Serup 1987)* and by thermography *(Agner & Serup 1988a)* after exposure to various irritants. Functional differences related to the chemical properties of the damaging irritant have also been reported. *Thiele & Malten (1973)* found differences in the electrical behaviour of the skin after exposure to different irritants, and *van der Valk et al (1985a)* reported that the relationship between barrier function impairment and damage to the viable cells of the deeper layers of the skin depends on the irritant used. Similarly, we found that SLS impaired barrier function to a greater extent than nonanoic acid or hydrochloric acid, although causing identical severity of the inflammatory response *(Agner & Serup 1989 (I))*. This property of SLS makes the irritant an interesting tool when studying the water barrier of the skin.

The kinetics of the skin response varies for different irritants. Generally, after removal of the irritant the skin response gradually becomes weaker. Examples of chemicals known to cause delayed irritation are hexanediol and butanediol diacrylate *(Malten et al 1979)*. SLS has been reported to cause a delayed response *(Dahl & Trancik 1977, Bruynzeel et al 1982, Agner & Serup 1989 (I))*. *Tupker et al (1990a)* found that the time course of transepidermal water loss after a 24 h SLS patch varies between different subjects. Using SLS in varying concentrations, *Serup & Staberg (1987a)* found a delayed response only for reactions clinically scored as 1+, but not for more intense reactions, indicating that the kinetics of the response may depend on the severity of the reaction *(Staberg & Serup 1988)*.

Dose. The skin response depends upon the concentration of the irritant applied *(Agner & Serup 1990 (IV))*. High concentrations may lead to severe inflammatory reactions in all subjects, leaving no opportunity for ranking skin susceptibility or for differentiating between reactions elicited by different irritants. Weak concentrations may induce no response at all. For SLS, a threshold concentration leading to a visible response in some, but not all, subjects was found useful *(Agner 1991 (VII, VIII, IX))*. The concentration of the irritant should also be chosen in accordance with the test region, climate and season, expected sensitivity of the test panel, patch test system and volume of irritant applied to the skin.

Quantitative differences in skin responses to different qualities of SLS. SLS has been used in widely varying concentrations in human volunteers (Table 2), and though some of the variation can be explained by different test techniques, the variation in concentration has caused some confusion, and made comparison of results difficult. We found significant differences in the irritant potential in vivo for different qualities of SLS *(Agner et al 1989 (II))*. High-performance liquid chromatography (HPLC) analysis of different SLS qualities revealed that in some SLS qualities part of the C_{12} chains had been substituted by longer C chains *(Agner et al 1989 (II))*, which are known to be less irritating to the skin *(Kligman & Wooding 1967, Stillman et al 1975)*. The differences in skin response to analogous concentrations of SLS found in the literature may well be explained by discrepancies in the amount of C_{12} chains in the different SLS products. Use of well-defined high-purity test substances is necessary to generalize and compare obtained responses.

Effect of vehicle. The vehicle may sometimes enhance the skin response, as demonstrated for urea *(Agner, in press)*, and in some instances cause irritation by itself, as found for propanol *(Agner & Serup 1987)*. Dimethyl sulfoxide (DMSO) is a potent enhancer which also causes significant irritation *(Kligman 1965, Baker 1968, Agner & Serup 1989)*. The vehicle may also influence the percentage of

SLS released from the test chamber system *(Agner et al 1990a)*.

NONINVASIVE MEASURING METHODS

During the last few decades several measuring devices designed for noninvasive investigation of the skin have become commercially available *(Berardesca & Maibach 1988a)*. Together these devices are called "bioengineering methods". The purposes of using these methods is to quantify reactions and, for some of the methods, to obtain information which is not detectable by the clinician's eye and finger. Because of their noninvasive approach, these techniques allow follow-up examinations. Introduction of noninvasive methods into the study of irritant contact dermatitis has opened up a new field of investigation. However, to evaluate the usefulness of each method, knowledge of variation and limitations of the methods must be taken into account, and their value in clinical and experimental settings assessed.

In the following, only methods closely related to the author's study will be discussed.

Measurement of transepidermal water loss (TEWL)

Water is lost through the skin in 2 ways, eccrine sweating and transepidermal diffusion, the latter representing the passive diffusion of water through the stratum corneum, commonly known as transepidermal water loss. The Evaporimeter (Servo Med^{R} EP1, Stockholm, Sweden) records the total evaporation from the skin and, since sweating should be suppressed during measurements, the term transepidermal water loss is now generally accepted for the recording of this instrument. The diffusion rate depends directly upon the ambient relative humidity (RH), ambient and skin temperature, and the integrity of the stratum corneum. Examination of the day-to-day intra-individual variation in TEWL indicates that baseline TEWL is a stable personal characteristic, when studied over a period of 10 days under standardized environmental conditions *(Pinnagoda et al 1989b)*.

A boundary layer about 10 mm in thickness develops around the skin, in which a water vapour gradient exists between the skin surface and the ambient air. The sensors of the Evaporimeter, mounted in the open chamber of the probe, determine the water vapour pressure gradient of this boundary layer, in order to quantify the diffusion of water through the skin, i.e. the TEWL. In the open-chamber water-vapour-pressure-gradient estimation method, a continuous measurement in ambient air, with little alteration of the vapour boundary layer, is provided, and this method is therefore preferable to the previously used closed chamber methods, which are incapable of continuous measurements and tend to interfere with the spontaneous TEWL *(Pinnagoda et al 1990)*.

Since the TEWL is estimated as a humidity gradient, the relative humidity in the measuring atmosphere will influence the TEWL *(Bettley & Grice 1967, Hattingh 1972, Pinnagoda et al 1990)*. In the Danish climate, higher TEWL values should be expected in the winter, when the relative humidity is low, as compared to values obtained in the summer season with high humidity, although the difference for basal values in our study was found not to be statistically significant *(Agner & Serup 1989 (V))*.

Changes in skin temperature modify the diffusion coefficient of the stratum corneum and rapidly lead to changes in TEWL *(Hattingh 1972, Mathias et al 1981)*. A formula for conversion of the TEWL to a standard temperature, considering the exponential relationship between TEWL and skin temperature, was first given by *Grice et al (1971)* and later modified by *Mathias et al (1981)*. None of these calculations were however based on results obtained with the open ventilated chamber technique. We found that the skin temperature of the participants after a period of rest at 20°C room temperature was very consistent within the range 29-31°C. Skin temperature being limited within this narrow range, conversion of TEWL values to match a standard temperature may introduce greater variables than the one introduced by the variation in temperature itself.

Eccrine sweating will generally not disturb measurements of TEWL on the trunk or on the limbs after a period of rest about 15 min at 20°C ambient temperature with the skin uncovered *(Baker & Kligman 1967, Coenraads et al 1986)*. Comparison of TEWL values obtained from normal skin and from skin exposed to scopolamine hydrobromide, for the purpose of sweat gland inactivation, revealed no differences in most subjects *(Pinnagoda et al 1989c)*. Nevertheless, a few people may still be sweating after a resting period, probably due to emotional stress during the measurement. Sweating shows up since stable values cannot be obtained if eccrine sweating is present. A prolonged resting period or "dummy measurements" to make the subject familiar with the measuring situation may be helpful, since it has been demonstrated that the first measurement in most individuals shows more fluctuations in TEWL than any subsequent *(Pinnagoda et al 1989b)*. In the author's experience "emotional sweaters" constitute only a minor proportion of the population, around 1-2%. In these subjects sweat gland inactivation by scopolamine may be beneficial. In addition, measurement during the hot summer season and in workers in heavy industries is possible only if sweat glands are inactivated.

Draughts in the surrounding environment will disturb the boundary layer and the water vapour gradient around the skin, and hamper the acquisition of valid TEWL measurements. By employment of a draught shield we found the standard deviation of the measurments to decrease significantly *(Agner & Serup 1990b)*. Use of protection covers, which may be necessary for sanitary reasons and to protect the sensors from damage, decreases the level of the measurement, due to increased distance from the skin surface *(Agner & Serup 1990b)*. When comparing results from different laboratories it is therefore necessary to know the exact circumstances under which the measurements were made.

Assessment of skin damage. A positive dose-response relationship for skin response to SLS as measured by TEWL has been demonstrated *(Serup & Staberg 1987a, Wilhelm et al 1989, Agner & Serup 1990 (IV))*. TEWL values were reported to be increased in scaly eczema lesions when measured on diseased skin *(Blichmann & Serup 1987)*. TEWL was found to be increased both in dry and in clinically normal skin in patients with atopic dermatitis *(Werner & Lindberg 1985)*.

Electrical conductance and capacitance

Different techniques have been developed for noninvasive assessment of skin hydration *(Leveque & de Rigal 1983)*. The Skicon 100^R hydrometer, described by *Tagami et al (1980)*, measures skin conductance. The application system consists of 2 concentrically arranged electrodes separated by an isolator. The resistance to a high frequency current of 3.5 MHz is measured, and given as 1/μohm. The Corneometer CM 420^R (GMBH, Köln, FRG) measures the electrical capacitance of the outer epidermis *(Werner 1986)*. The probe of this instrument is a plastic-foil-covered brass grid, which functions as one electrode while the skin functions as the other. While the Skicon 100^R registers hydration in the outermost portion of the stratum corneum, the Corneometer CM 420^R registers hydration down to a depth of about 0.1mm *(Blichmann & Serup 1988)*. The Skicon 100^R was found to be more suitable for measurement of increased skin hydration, while the Corneometer CM 420^R was more sensitive for measurement of decreased hydration. The correlation between the 2 methods on normal skin was reported as R = 0.654 *(Agner & Serup 1988b)*.

Assessment of skin damage. When attempting to quantify irritant patch test reactions by measurement of electrical conductance, we found the intra-individual variation in the results to be so high that the method was found unhelpful for this purpose *(Agner & Serup 1990 (III))*. However, we studied only the initial inflammatory phase of the response, and conductance measurement may be useful to quantify dry and scaly stages occurring later. Decreased water binding in the stratum corneum in scaly hand eczema lesions, as assessed by measurement of electrical conductance and capacitance, has been demonstrated *(Blichmann & Serup 1987)*. The electrical capacitance of the stratum corneum of patients with atopic dermatitis and clinically dry skin was reported to be significantly lower than that in normal controls *(Werner 1986)*.

Laser Doppler flowmetry

Laser Doppler flowmetry is a noninvasive technique for direct measurement of blood flow. The technique combines the Doppler phenomenon with the spectral purity of laser light and the light beating technique *(Shepherd 1990)*. Monochromatic laser light is directed into the tissue via an optical fibre to a depth of about 1 mm. After partial absorption and diffuse scattering has taken place, the light is reflected with Doppler-shifted frequencies from moving blood cells in the skin and with unshifted frequencies from stationary tissues. The result is read from the instrument in arbitrary units.

The conventional probe holder of the Periflux^R (Perimed, Stockholm, Sweden) laser Doppler is attached to the skin by adhesive tape. Repeated applications of the probe, which are helpful in studies of inflammation due to spatial variation in blood flow, will influence the test results through irritation. A plastic block with a hole for the probe, positioning the fibres just above the skin, and Velcro^R straps for fastening around the arm, was in our studies used to obtain a stable positioning of the probe without the use of adhesive tape *(Agner & Serup 1990 (IV))*. In order to diminish variation due to local changes in the microcirculation, a probe holder with a distance between the skin and the probe of 4 mm has been proposed as allowing for a larger area of investigation *(de Boer 1989)*. However, a need for physical contact between the probe tip and the skin was argued by *Nilsson (1990)*, in order to avoid relative motion between them and to prevent scattering of light in the tissue surface directly into the fibres.

Assessment of skin damage. A positive relationship was found between applied dose of SLS and blood flow values recorded by laser Doppler flowmetry *(Nilsson et al 1982, Staberg & Serup 1988, Agner & Serup 1990 (IV))*. Also, a close correlation between mean blood flow values and visual scoring in a study with 7 different irritants has been reported *(Willis et al 1988a)*. However, wide fluctuations in laser-Doppler-recorded blood flow values in response to SLS patches were found due to spotty erythema *(Freeman & Maibach 1988)*. *Blanken et al (1986)* found no dose-response relationship for blood flow in 0.5-2% SLS patches assessed by laser Doppler flowmetry in 24 healthy subjects. In that study only one measurement was obtained from each test site. Due to local variation in the microcirculation an average of a number of measurements will give more valid results.

Colorimetry

The skin surface colour can be quantified using the standard tristimulus system suggested by the Commission Internationale de l'Eclairage (CIE) *(Robertson 1977)*. The colour assessment is adjusted to the nonlinear perception of colour by the human eye, and the idea is to replicate colour as it is acknowledged by the human eye and brain. Colour measurement by the colorimeter Minolta Chroma Meter CR-200 (Osaka, Japan) is based on illumination of the skin by xenon flash light. The colour is expressed in a 3-dimensional coordinate system (Fig.1). Luminance (L*) expresses the brightness (integrated reflection of light from the surface), ranging from total black (low values) to pure white (high values). The a* and the b* are the two colour coordinates: a* represents the colour range from green (-) to red (+), and b* the colour range from blue (-) to yellow (+). The "true" colour of the skin is expressed as an admixture of the a*, b* and L* values.

Although the a* value is an indicator of the presence of

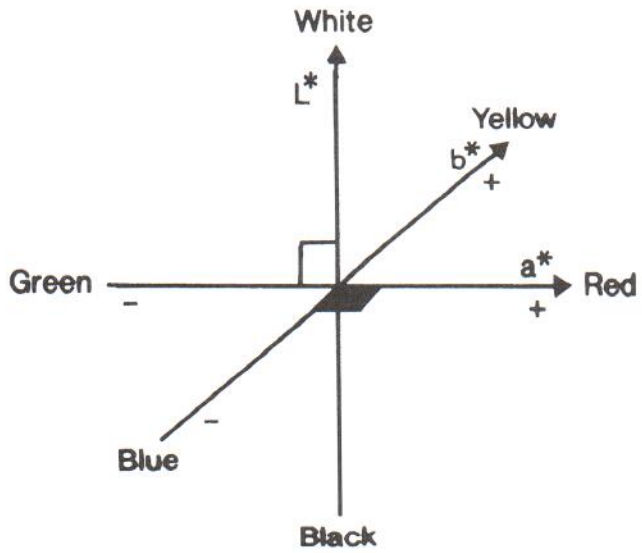

Fig.1. Schematic drawing indicating the main principles of the L*a*b* colour system. The a*-axis represents the colour range from green (-) to red (+) and the b*-axis represents the colour range from blue (-) to yellow (+). L* expresses the brightness (reflection of light) ranging from black (low values) to white (high values).

haemoglobin, it is also influenced by other chromophores in the skin (melanin, haemoglobin, bilirubin, carotene), and by structural conditions in the skin. The light reflection from the skin surface (L*) is also a complex factor, influenced not only by chromophores in the skin but also by factors which will change the skin surface reflectiveness, like skin dryness or roughness, and by structural differences in the epidermis and dermis. Another method for quantification of erythema, based on comparison of the amount of reflected green and red light from the skin, has been proposed: by this method a recorded change in erythema is essentially related to an increase in vasodilatation and is largely independent of the melanin content of the epidermis *(Diffey et al 1984, Farr & Diffey 1984)*. A more specific determination of skin colour relative to its pigments is thus theoretically possible, and instruments based on this principle have recently become commercially available.

Since redness of the skin is easily influenced by skin temperature and emotional or physical stress a period of rest before measurement is obligatory. Changes in the pressure of the probe against the skin may influence the measuring results if the probe is not attached gently.

Assessment of skin damage. a* colour coordinates have been demonstrated to correlate well with visual scoring in inflammatory reactions caused by soap or SLS *(Babulak et al 1986, Serup & Agner 1990)*. A positive correlation between changes in the a* colour coordinates and doses of SLS has been reported *(Wilhelm et al 1989, Agner & Serup 1990 (IV))*. Measurement of integrated reflection of light has been used to quantify blanching *(Queille-Roussel et al 1990a)*, and for assessment of constitutional skin colour *(Westerhof et al 1990)*. *Seitz & Whitmore (1988)* found that a* was significantly correlated with the dermatologist's perception of erythema, b* was significantly associated with the perception of tanning after UV exposure, while decreased L* values were associated with the clinical perception of erythema as well as tanning. Due to repeated UV exposure during the experiment, it was however difficult to differentiate between changes in skin colour caused by inflammation and by tanning *(Seitz & Whitmore 1988)*.

Ultrasound A-scan

High frequency ultrasound for skin examination was introduced by *Alexander & Miller (1979)*. Today, A-scan for 1-dimensional, B-scan for 2-dimensional and C-scan for 3-dimensional study of the skin are commercially available *(Serup, in press)*. A-mode scanning by the Dermascan A^R (Cortex technology, Denmark) has been reported as an accurate technique for the measurement of skin thickness *(Serup et al 1984, Agner & Serup 1990 (III))*. The interval between the echo from the skin surface and the echo from the interface between the dermis and subcutaneous fat is a measure of the thickness of the skin (i.e. epidermis and dermis, Fig. 2). The distance in millimeters can be calculated from the sound velocity in the tissue, reported as 1580-1605 m/sec in skin *(Edwards & Payne 1984, Escoffier et al 1986)*. The dermis is highly echogenic in most body regions, and oedema formation in the dermis leads to a zone with very few and low echoes. In most parts of the body the differentiation between epidermis and dermis by ultrasound is difficult. An exception is the palm. At 10 MHz the subcutaneous area has a high resolution, while at 50 MHz the epidermis can be studied more in detail. With respect to the compromise between resolution and depth of the viewing field, 20 MHz has been established as a good solution.

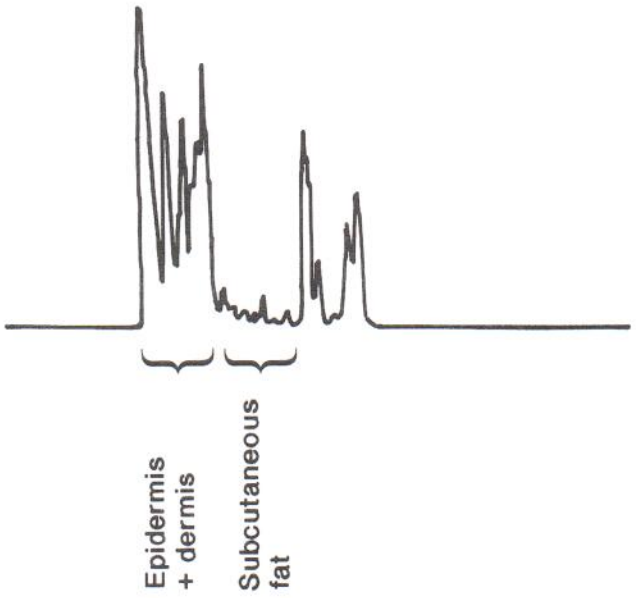

Fig.2. Schematic drawing of an ultrasound A-scan from normal skin.

The interface between the dermis and the subcutaneous fat is far from flat. This inherent variation in the biology limits the precision of thickness measurements. However, if 3 or more sites are examined and averaged the reproducibility of ultrasound A-mode skin thickness measurement is high *(Agner & Serup 1990 (III))*. By the use of the Dermascan^{R} C-mode scanner, mean values can be calculated from 224 A-mode lines covering a 22.4 mm lateral scanning field. This is probably now a more accurate way of measuring skin thickness, since biological variation in the dermis/subcutaneous fat interface is overcome.

Assessment of skin damage. Ultrasound A-scan has been found suitable for quantification of patch test reactions *(Serup et al 1984, Serup & Staberg 1987b)*, although some authors, probably due to the use of less-developed equipment, did not find the method useful *(Brazier & Shaw 1986)*. Compared to other bioengineering methods, ultrasound examination has the advantage that no preconditioning of the subjects is necessary before measurement. Modest changes in ambient temperature, relative humidity, air convection, disturbances in the room during measurement and emotional status will not influence the measurement.

Comparison of noninvasive techniques for assessment of SLS-induced skin damage

Comparing evaporimetry, laser Doppler flowmetry, ultrasound A-scan and measurement of skin colour (a*), evaporimetry was found to be the best suited method overall for evaluation of SLS-induced skin damage *(Wilhelm et al 1989, Agner & Serup 1990 (IV))*. Colour measurement by the Commission Internationale de l'Eclairage (CIE) system, as indicated by a change in the a* colour coordinate, was consistently the least sensitive of the methods, though the usefulness of the instrument has been advocated by *Wilhelm et al (1989)*, who emphasize the convenience of the apparatus. The sensitivity of skin blood flow measurement or skin thickness measurement varied according to dose of SLS and interval between removal of patch test and evaluation *(Agner & Serup 1990 (IV))*. The order of the methods for assessment of SLS-induced skin damage estimated from our dose-response study *(Agner & Serup 1990 (IV))* was confirmed in subsequent clinical studies, where colour (a*) measurement and laser Doppler flowmetry failed to differentiate between reactions which could be differentiated by measurement of TEWL and skin thickness *(Agner 1991 (VIII,IX))*. These conclusions on the measuring methods are limited to SLS-induced irritation. Other irritants may affect the skin in different ways and may give rise to a different order of preference of techniques *(Queille-Roussel et al 1990b)*.

SKIN IRRITATION AND SENSITIVE SKIN

The skin as a barrier. In its role as a barrier, the skin participates in homeostasis by limiting water loss from the inside to the outside and limiting percutaneous penetration of environmental agents from the outside to the inside, but also in a broader sense by resisting chemical and physical trauma. Implications of proper function of the barrier thus reach further than avoidance of skin diseases. Definition and localization of the barrier depends upon the character of the barrier addressed. In the present review, the expression skin barrier refers only to the water permeability barrier, localized in the stratum corneum *(Baker & Kligman 1967)*.

Sensitive skin. Exposed to the same exogenous conditions, some subjects will develop an irritant eczema while others will not. The group which develop eczema may be expected to have an increased skin susceptibility/increased skin reactivity or more sensitive skin than the rest. Whether the concept "sensitive skin" in fact exists has been debated. In his pioneering study of primary irritants, Björnberg found no correlation between the intensity of skin responses evoked by 11 different primary irritants, and stated that the response to one particular irritant does not necessarily predict the response to another irritant *(Björnberg 1968, p 140)*. Patients with atopic dermatitis and pompholyx were excluded. Björnberg's findings were supported by later studies *(Coenraads et al 1975, Wahlberg et al 1985)*.

In contrast to these findings, a statistically significant correlation between the skin response to particular irritants was reported in healthy volunteers and patients with various skin diseases *(Frosch & Kligman 1977)*, although the correlation in most situations was only moderate *(Frosch 1985, p 78)*. A group of individuals with sensitive skin could be identified by assessment of susceptibility to skin tests with 7 different irritants and assessment of minimal erythema dose (MED) *(Frosch & Wissing 1982)*. For pre-selection of "hyperreactors", *Frosch & Kligman (1979a)* for practical reasons used a 24 h forearm chamber exposure to 5% aqueous SLS, but suggested that a series of tests should preferably be used to identify "members of the delicate skin

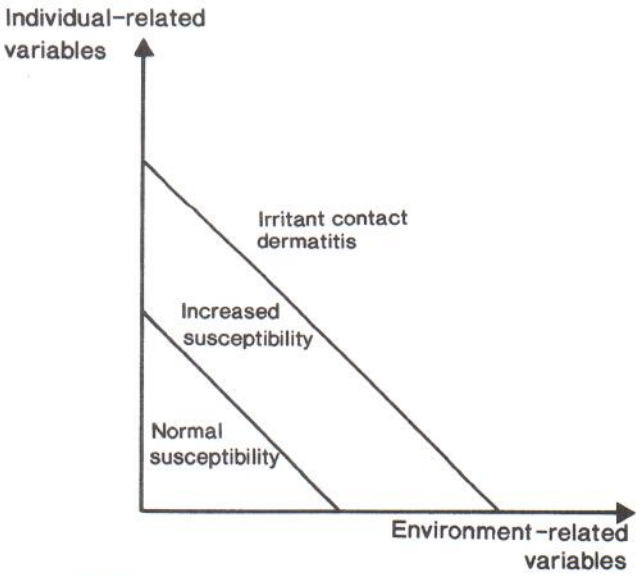

Fig.3. A combination of individual- and environment-related variables determines the current susceptibility of the skin.

club" *(Frosch & Kligman 1982)*. When testing chemically similar substances the same ranking order was found in most individuals *(Tupker et al 1989a)*.

In a limited number of subjects we found a positive correlation between the inflammatory response to SLS and hydrochloric acid, while the response to nonanoic acid did not correlate with that of other irritants *(Agner & Serup 1989 (I))*.

The contradiction between reports that no correlation between the intensity of skin responses evoked by different irritants exists, and statements that "hyperreactors" to irritants can be identified, may partly be explained by choice of irritants, dose, test region and test method. Different penetration abilities of particular irritants may account for discrepancies in the intensity of the evoked skin response, since irritants penetrating the barrier at a fast rate may be less dependent on individual barrier function than irritants penetrating at a slow rate. Use of high doses of irritants, eliciting severe reactions, may tend to equalize skin responses. Regional variations in skin susceptibility may exist even within narrow anatomical areas and influence the response *(Flannigan et al 1984)*. The chamber technique may be more suited for irritant testing than the previously used test methods.

Irritant contact dermatitis. Irritant contact dermatitis is a complex disease, with a multifactorial pathogenesis, to which environmental as well as individual factors contribute (Fig. 3). Within the individual, the response to irritant stimuli depends on the skin barrier function, the inflammatory reactivity of the skin and – addressing chronic irritant contact dermatitis – its regeneration ability. Cumulative barrier function impairment may finally lead to irritant contact dermatitis *(Malten 1981)*. In our experimental studies, the magnitude of skin response to a single challenge with SLS was used for determination of skin susceptibility. SLS has the ability to penetrate and impair the barrier, and thus reflects the barrier function. The function of the barrier is important for the irritant effect of many chemical substances, since they have to penetrate the barrier to reach the viable cell layers and elicit an inflammatory reaction. The skin response to a 24 h SLS patch test is however also influenced by the inflammatory reactivity of the skin, and will reflect the current state of this.

Physiological variation in skin susceptibility in healthy subjects

Exposed to low grade irritancy products, only a minor proportion of a population will develop irritant contact dermatitis, while the majority will remain free of symptoms. This variation in skin response may to some extent be explained by inherent structural differences in the skin of the individual, and to some extent by exogenous conditions to which the skin is/has been exposed.

Some individual- and environment-related variables influencing skin susceptibility will be discussed in the following.

Individual-related variables: Clinical

Sex. Hand eczema is known to occur more frequently among women than among men *(Rystedt 1985a, Meding & Swanbeck 1989)*. This might however reflect differences in exposure to environmental hazards rather than endogenous differences between the sexes. Most investigations have found no sex-relation in skin susceptibility *(Bjørnberg 1975, Kligman 1980, Lammintausta et al 1987b, Tupker et al 1989b)*. In one study the impression that female skin was more easily irritated, as evaluated by the increase in TEWL after SLS-exposure was reported, but no statistically significant difference in total TEWL after SLS-exposure was found *(Goh & Chia 1988)*. Throughout our studies no sex-related difference in skin susceptibility to irritants was found.

Menstrual cycle. Increased reactivity of the skin prior to and during the menstrual phase was reported by *Halter (1941)*, and Björnberg avoided testing females prior to and during menstruation *(Bjørnberg 1968, p 29)*. Newer casuistic reports support the impression of changes in skin reactivity during the menstrual cycle *(Alexander 1988, Kemmett 1989)*. Estradiol has been reported to suppress the cellular immune response *(Myers et al 1986)*, but the significance for irritant contact dermatitis is not known. We found increased reactivity to SLS at day 1 in the menstrual cycle as compared to days 9-11, when tested on opposite arms in healthy women *(Agner et al 1991 (VI))*, (Table 3). The theoretical possibility that our results may have been influenced by a hyporeactive state *(Lammintausta et al 1987a)* induced by repeated exposure to SLS, though different and distant skin areas were exposed, is opposed by the lack of variation in our male control group *(Agner et al 1991 (VI))*. Since no cyclical variation was found in baseline TEWL, the increased reactivity of the skin at day 1 in the menstrual cycle probably reflects an increased inflammatory reactivity, rather than changes in the barrier function. Our observations confirm the clinical impression and indicate that the menstrual cycle should be considered in the discussion of skin reactivity.

Age. Increased susceptibility to DMSO in childhood was reported by *Frosch (1985, p 39)*. Increased susceptibility to SLS in young compared to elderly females, when assessed by visual scoring and TEWL, was reported *(Cua et al 1990)*, and the increase in TEWL values was found to be more persistent in the older group *(Elsner et al 1990)*. These findings imply less reaction to an irritant stimulus but a prolonged healing period in older people. Within the 18-50 year range no significant influence of age on skin susceptibility should be expected *(Kligman 1980, Agner 1991 (VII))*.

Regional differences. Susceptibility to exogenous irritant stimuli differs between anatomical regions. Benzalkonium chloride on cellulose patch tests was reported to elicit stronger reactions on the back than on the extremities *(Magnusson & Hersle 1965b)*, though the pressure of cloth-

Table 3. *SLS-exposed skin at different times in the menstrual cycle*

SLS-EXPOSED SKIN	1. TEST	2. TEST	p
TEWL	15.8 (12.1-20.0)	13.7 (11.0-15.9)	$p<0.05$
Blood flow	24 (14-40)	17 (9-36)	n.s.
Skin thickness	1.23 (1.18-1.34)	1.15 (1.04-1.25)	$p<0.005$

Median values and 25/75 percentiles of transepidermal water loss (TEWL, g/m²h), superficial blood flow (arbitrary units) and skin thickness (mm) as measured on skin after exposure to sodium lauryl sulphate for 24 h. Values for day 1 (1. test) as well as days 9-11 (2. test) in the menstrual cycle are given. n = 29 females. n.s. indicates $p>0.05$.

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ing on the patches may have influenced the results *(Gollhausen 1985)*. Skin susceptibility to ammonium hydroxide was reported to be ranked as forearm < leg < back < postauricular skin *(Frosch & Kligman 1977)*, and susceptibility to DMSO to be ranked as leg < forearm < back < forehead *(Frosch et al 1980)*. Percutaneous absorption of benzoic acid, as a reflection of barrier function, was studied at different regional anatomic sites in man, and the permeability in different regions was ranked as back < arm < chest < thigh < abdomen < forehead *(Dupuis et al 1986)*. A linear relationship between TEWL and penetration was reported in the same study. Baseline TEWL with respect to anatomical sites can be ranked as follows: back = abdomen = chest = thigh = arm < dorsum of hand < postauricular = forehead < sole < palm *(Pinnagoda et al 1990)*. However, the linear relationship between TEWL and penetration of exogenous substances cannot be generalized to all anatomic sites and every substance. The described regional variation in skin barrier function may depend on the irritant applied, the ranking order of the different anatomical regions being different for different chemicals. The absorption of hydrocortisone from the palm was reported as 0.83% of applied dose as compared to 1% from the arm *(Feldmann & Maibach 1967)*, though the baseline TEWL of the palm is higher by a factor of 6-7 compared to the arm *(Pinnagoda et al 1990)*. Although the TEWL values reported from the palms and soles may be biased by the high density of sweat glands in this area, the water loss from the nail is also reported to be significantly increased as compared to forearm values *(Jemec et al 1989)*.

Thus, major differences in skin barrier function with respect to anatomical sites exist.

Individual-related variables: Experimental

Baseline transepidermal water loss. The use of baseline TEWL to predict the skin response to surfactants was suggested by *Murahata et al (1986)*. Healthy subjects were tested with a standard soap chamber irritation insult, and high baseline TEWL was found to be associated with high susceptibility to soap irritation, as evaluated by visual scoring. This relationship between skin susceptibility to detergents and high baseline TEWL was further supported by *Tupker et al (1989b and 1990b)*, and a highly significant correlation between baseline TEWL and TEWL after a single or repeated exposure to SLS was reported by the same group *(Pinnagoda et al 1989a)*. In our study of healthy subjects challenged with SLS, baseline TEWL was found to contribute significantly to a multiple regression analysis model, using TEWL after exposure to SLS as the dependent variable *(Agner 1991 (VII))*, (Table 4). Persons with high visual scores after SLS-exposure had increased basal TEWL as compared to those with low visual scores *(Agner 1991 (VII))*. However, some studies report an absent or poor correlation between baseline TEWL and TEWL after SLS-exposure *(Berardesca & Maibach 1988 b + c, Freeman & Maibach 1988, Wilhelm & Maibach 1990 + 1991)*. Differences in measuring situations may have influenced the results, since optimal measuring conditions are necessary for detection of small differences in TEWL values *(Pinnagoda et al 1990)*.

Repetitive measurements of baseline TEWL in workers in the metal industry in Singapore indicated that pre-employment TEWL measurements from the backs of the hands may predict later development of irritant contact dermatitis in high-risk professions *(Coenraads & Pinnagoda 1985, Coenraads et al 1986)*. Although promising, these results can only be accepted as preliminary, since the measurements were obtained under poorly standardized cir-

Table 4. *Multiple regression analysis. TEWL after SLS exposure as dependent variable*

Independent variables	p-value (exclusion)	regression coeff.
Constant term		- 35.136
Basal TEWL	< 0.00005	4.013
L*	< 0.001	0.459

N = 70 R-square = 0.659

The independent variables included in the model are listed in the left column. L* = luminance (white is indicated by a high number and black by a low number). The remaining independent variables: basal skin thickness, basal skin blood flow, the basal colour coordinates a* and b*, sex and age did not contribute statistically significantly to the model, and were thus not included. The contribution of each independent variable to the model is indicated by the regression coefficient, and the statistical significance by a p-value. The R-square value represents the amount of the total variation in data explained by the model.

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cumstances on an ethnically heterogenous group, only 4 subjects developed dermatitis, and 22% of the workers were not followed up. The study also indicated an increase in TEWL in parallel with stratum corneum damage in subjects at risk for subsequently developing irritant contact dermatitis *(Coenraads et al 1986)*. An increased basal TEWL may thus reflect a constitutionally impaired barrier function, an impaired barrier function due to exogenous exposure, or both, the difference between these two conditions being mainly of theoretical concern (Fig. 3). Measurement of baseline TEWL at selected sites (e.g. dorsum of the hand) might be valuable in evaluating the current risk for surpassing a critical level, in consequence of which a clinical disease, irritant contact dermatitis, develops.

Skin hydration. In a set-up with repetitive exposures to SLS, higher susceptibility was reported in dry skin than in clinically normal skin in eczematous subjects and controls *(Tupker et al 1990b)*. In the same study, skin dryness as a dichotomous variable contributed significantly to a multiple regression analysis model, using TEWL after exposure to SLS as the dependent variable, while baseline hydration measured as a continuous variable by the Corneometer did not contribute significantly. In contrast to this, *Lammintausta et al (1988)* found no relationship between clinically dry skin and the response to repeated SLS exposure. Comparing winter and summer skin in a paired design, decreased skin hydration was found in winter, when a higher reactivity towards SLS was also found *(Agner & Serup 1989 (V))*. A negative correlation between skin hydration and TEWL has been reported in normal skin and various skin diseases *(Werner & Lindberg 1985, Blichmann & Serup 1987, Tagami 1990)*, though this was not found by *Tupker et al (1990b)*.

Thus, some studies indicate that a decreased hydration state of the skin may be associated with impaired barrier function and increased skin susceptibility.

Skin blood flow. Assessment of the relationship between basal skin blood flow and skin susceptibility has not been the subject of much investigation. In our study on healthy volunteers we found that basal blood flow as measured by laser Doppler flowmetry did not contribute significantly to a multiple regression analysis model, using TEWL after SLS-exposure as the dependent variable *(Agner 1991 (VII))*.

Skin colour. Fair skin and blue eyes were reported to correlate with the intensity of the inflammatory response to a mechanical irritant *(Björnberg et al 1979)*. By determination of MED in Caucasian volunteers, the cutaneous sensitivity to UV light and to 7 different chemical irritants was found to correlate positively, while skin typing based on complexion and history of sunburn proved less reliable *(Frosch & Wissing 1982)*. In contrast to these reports, an inclination toward increased susceptibility to SLS in black and Hispanic skin as compared to white skin was found when evaluated by measurement of TEWL, though the differences were statistically significant only between pre-occluded black and white skin using 0.5% SLS *(Berardesca & Maibach 1988 b+c)*. Differences in black and white human skin have previously been reviewed: it was concluded that the difference in irritancy response between the 2 races might be less pronounced than previously reported, due to difficulties in detecting erythema on black skin *(Andersen & Maibach 1979)*.

Assessing skin colour by a tri-stimulus colorimeter, we found an association between increased light reflection from the skin surface ("fair" skin) and increased susceptibility to SLS *(Agner 1991 (VII))*, (Table 4). This finding supports the assumption of an association between skin colour and skin susceptibility. However, reflection of light (L*) indicates presence of skin chromophores as well as nonspecific structural parameters, and a more specific determination of skin colour, relative to its pigments, could probably be obtained using an instrument based on the principles of *Farr & Diffey (1984)*.

Tanning may influence the susceptibility to irritants. An increased blister formation time after exposure to ammonium hydroxide has been demonstrated in tanned skin *(Frosch & Kligman 1977)*. A diminished reaction to SLS after UVB exposure was reported *(Larmi et al 1989)*. This change in skin reactivity may reflect other modifications in the skin induced by UV light, apart from tanning.

The background for the relationship between fair and sensitive skin is however not well understood, since structural differences other than melanin should be considered. An association between black skin and an increased number of cell layers in the stratum corneum as compared to white skin has been reported *(Weigand et al 1974)*, while *Freeman et al (1962)* reported of no significant difference in stratum corneum thickness between subjects with fair or dark complexion, nor between black and white skin. In vitro baseline TEWL was reported to be higher in black than in white skin *(Wilson et al 1988)*, but in vivo no significant difference in baseline TEWL was found between black and white skin, nor between Hispanic and white skin *(Berardesca & Maibach 1988 b+c)*. However, an objective measurement of skin colour may, among other determinants, be useful for assessing individual skin susceptibility.

Skin thickness. The importance of the thickness of stratum corneum for skin susceptibility was argued by Frosch, who found that the minimal blistering time after exposure to ammonium hydroxide was directly related to the number of cell layers in the stratum corneum *(Frosch & Kligman 1977)*, and the response to DMSO was increased after stripping of the stratum corneum *(Frosch et al 1980)*. However, penetration of water was reported not to be influenced by the number of cell layers or thickness of the stratum corneum, while the total lipid concentration of this layer was found to be a critical factor *(Elias et al 1981)*.

By ultrasound A-scan the measurement of skin thickness includes the epidermis together with the dermis, the latter constituting the major part of the measured distance (Fig. 2). Alterations in the thickness of the dermis may therefore easily be detected, while the same percentage of alteration

in the thickness of the epidermis may be beyond the detection limit. In the study on normal skin, basal skin thickness as measured by ultrasound did not contribute as a risk factor for skin susceptibility to SLS *(Agner 1991 (VII))*.

Environment-related variables

Seasonal variation. Seasonal variation in contact dermatitis, due to variation in reactivity to irritant stimuli and due to variations in the occurrence of different allergens, has been described *(Hjorth 1967)*. Significantly increased skin response to DMSO was found in the winter compared to the summer *(Frosch 1985, p 39)*, and the same variation was demonstrated for propylene glycol *(Warshaw & Hermann 1952, Hannuksela et al 1975)*. *Frosch and Kligman (1979a)* stated that soap testing has greatest sensitivity in the winter, when the damaging action of soap is at its peak. We found a statistically significant seasonal variation in healthy volunteers exposed to SLS, known to damage the barrier function of the skin, while the seasonal variation for skin response to nonanoic acid, known to influence the barrier only to a minor degree *(Agner & Serup 1989 (I))*, was less pronounced *(Agner & Serup (V))*. Low outdoor temperature and low relative humidity in the winter leads to decreased ability of the stratum corneum to retain water *(Spencer 1975)*. The significance of relative humidity was highlighted by *Rycroft & Smith (1980)* in their report on low humidity occupational dermatoses. A significantly lower hydration state of the skin during winter than during summer was also demonstrated in our study *(Agner & Serup 1989 (V))*. The reports thus all confirm that seasonal variation in skin susceptibility exists.

Exposure to irritants. Environmental hazards are highly important for the development of irritant contact dermatitis. *Meding & Swanbeck (1990)* found that the type of hand eczema that is most dependent on occupation is irritant contact dermatitis. *Nilsson & Bäck (1986)* followed a population of 1857 women in wet hospital work for 20 months and found that the prevalence of hand eczema during the period was 41%. *Rystedt (1985a)* reported that endogenous factors (hand eczema in childhood, persistent eczema elsewhere than the hands, dry skin, severe childhood eczema, family history of atopy, concurrent asthma/rhinitis and female sex) together were of greater importance than exogenous factors (exposure to chemicals, water, soil and/or wear).

Pathophysiological conditions influencing susceptibility of uninvolved skin

Atopic dermatitis. The significance of a history of atopic dermatitis for the development of irritant hand eczema has been thoroughly demonstrated *(Rystedt 1985b + c, Nilsson & Bäck 1986, Meding & Swanbeck 1989)*.

In patients with atopic dermatitis the baseline TEWL was reported to be increased in both dry non-eczematous skin and in clinically normal skin on the forearm and on the back of the hand *(Werner & Lindberg 1985)*. This finding has been confirmed by others examining the uninvolved skin of patients with atopic dermatitis, on the forearm *(van der Valk et al 1985b, Tupker et al 1990b)* and on the upper arm *(Agner 1991 (IX))*. However, basal TEWL measured on the upper arm in hand eczema patients with a childhood history of atopic dermatitis, but without atopic manifestations in adult life other than hand eczema, was found to be normal *(Agner 1991 (VIII))*. Thus TEWL values may undergo changes related to time and to the course of the disease.

Clinically normal skin in patients with atopic dermatitis did not differ significantly from normal control skin with respect to electrical capacitance, when measured by the Corneometer CM420^{R} *(Werner 1986)*, and this finding was supported by measurement of electrical conductance *(Aljaberi & Marks 1984)*.

Although facial pallor is a common finding in atopic dermatitis *(Hanifin & Rajka 1980)*, no significant change in skin colour as measured by the colorimeter was found associated with atopic dermatitis *(Agner 1991 (IX))*.

An inclination toward increased basal skin thickness in clinically uninvolved skin in patients with atopic dermatitis was reported, but the difference was not statistically significant as compared to controls *(Agner 1991 (IX))*.

Tested in a quiet phase of the disease, patients with atopic dermatitis were found to react more severely to SLS than healthy controls as assessed by measurement of TEWL *(Van der Valk et al 1985b)*. We found an enhanced skin reactivity to SLS in patients with current atopic dermatitis, as compared to controls, confirmed statistically by visual scoring and by increase in skin thickness *(Agner 1991 (IX))*. Although higher TEWL values after SLS-exposure were found in atopic dermatitis patients, we found that the increase in TEWL was not significantly different from that in controls. In our study, occasional active eczematous lesions on skin distant from the test site may have influenced skin reactivity, a mechanism which is probably also highly important for the clinical course of the disease.

Hand eczema. Baseline TEWL measured on the forearm or upper arm in patients with localized, inactive or healed eczema is not reported to be significantly different from that in controls *(van der Valk et al 1985b, Agner 1991 (VIII))*. The question as to whether a local defect in the skin barrier (i.e. increased baseline TEWL) confined to the hand exists prior to the development of hand eczema, as indicated by the findings of Coenraads et al *(Coenraads & Pinnagoda 1985)*, still has to be answered. The significantly positive correlation between baseline TEWL and TEWL after exposure to SLS was confirmed in hand eczema patients *(Agner 1991 (VIII))*, (Fig.4).

Comparing patients with hand eczema to matched controls, increased L*-values and decreased b*-values measured by the colorimeter, indicating a fairer skin colour, were found *(Agner 1991 (VIII))*. This observation is in accordance with experimental findings of an association between fair complexion and sensitive skin *(Frosch & Wissing 1982, Agner 1991 (VII))*.

Basal skin thickness, as measured by ultrasound A-scan, was found to be significantly thinner in patients with hand

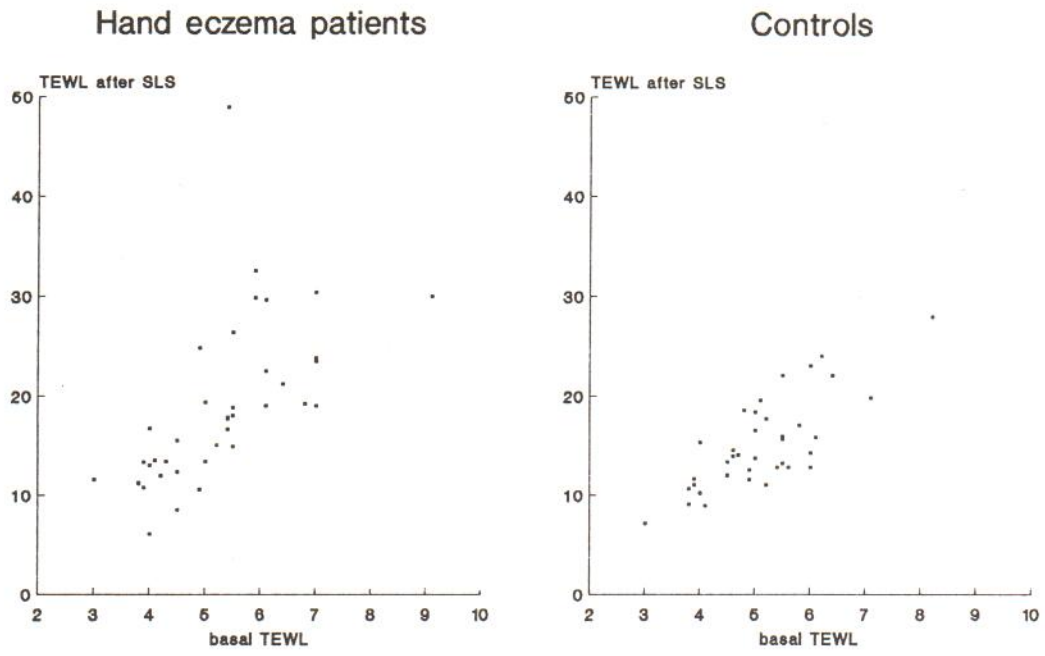

Fig.4. Correlation between basal TEWL and TEWL after exposure to SLS for patients with hand eczema (n = 39) and controls (n = 39). The Spearman coefficient of correlation was R = 0.79 ($p < 0.0005$) and R = 0.73 ($p < 0.0005$), respectively.

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eczema than in matched controls *(Agner 1991 (VIII))*. The interpretation of this result is difficult because use of topical corticosteroids on the hands might, at least theoretically, have influenced the skin on the upper arm. This finding might reflect a generalized structural anomaly in the skin of hand eczema patients, as might the above-mentioned change in skin colour. Further studies are necessary before a conclusion can be drawn.

Björnberg found that a constitutional increase in skin reactivity to primary irritants was not present in patients with healed hand eczema, when tested on skin distant from the eczema *(Björnberg 1968, p 139)*. *Van der Valk et al (1985b)* found no difference in TEWL after exposure to SLS in subjects with healed contact dermatitis, as compared to controls. In the author's study no increased skin reactivity to SLS in patients with chronic or healed eczema was found, as compared to controls, while hand eczema patients with acute eczema showed an increased skin reactivity to SLS compared to controls *(Agner 1991 (VIII))*. Thus, although hand eczema patients were found to be more "fair-skinned" than controls *(Agner 1991 (VIII))*, this factor alone did not influence the skin response to SLS significantly.

Hyperirritable skin

The phenomenon of dermatitis in a localized area of the body resulting in generalized hyperirritability of the skin, including areas distant from the eczema, is well known, though different terms have been used to describe it. "Conditioned hyperirritability", "auto-eczematization", and "status eczematicus" are among the terms used *(Roper & Jones 1982)*. Mechanisms responsible for the fluctuations in skin reactivity were recently discussed *(Bruynzeel & Maibach 1991)*. *Björnberg (1968)* demonstrated that dermatitis of the hands may have an enhancing effect on irritant patch test reactions elsewhere on the body. Increased susceptibility to SLS, benzalkonium chloride and hydrochloric acid tested on the thigh was found in patients with active localized hand eczema, when compared to healthy controls and to patients with healed eczema.

Mitchell (1977) introduced the term "angry back", describing the phenomenon of a single strong positive patch test reaction creating a back which is hyperreactive to other patch test applications. The excited skin syndrome, as it has also been called, was illustrated experimentally in guinea pigs: increased susceptibility to an ointment containing 1% SLS was observed in animals stressed by inflammatory reactions in the neck area *(Andersen & Maibach 1980)*. *Kligman & Gollhausen (1986)* were unable to demonstrate experimentally that a single positive patch test creates an angry back, and suggested that the described hyperirritability of the skin was due to preexisting dermatitis.

An increased susceptibility to SLS in patients with acute hand eczema, as compared to patients with chronic or healed eczema, was confirmed in our study *(Agner 1991 (VIII))*. While we found no difference between subjects with chronic/healed eczema and controls with respect to skin susceptibility to SLS, *Bruynzeel et al (1983)* reported a higher score to SLS in patients with inactive dermatitis than in subjects without dermatitis. The difference may be related to the criteria used to define "inactive" dermatitis.

Bruynzeel et al (1983) attempted to use SLS patches as markers of hyperirritability, but rejected it because no correlation between false positive reactions to allergic patch tests and the score of the SLS test was found. The use of SLS as a marker of hyperirritable skin was found useful in our study *(Agner 1991 (VIII))*. However, the skin response to SLS depends not only on the current inflammatory reactivity of the skin, but also on the skin barrier function.

Whether the skin barrier is generally influenced by the presence of active eczema is an open question. *Shahidullah et al (1969)* reported increased TEWL values in the clinically normal skin of patients with eczema. We found that basal TEWL values in patients with acute eczema were not statistically significantly higher than in controls, though there was a tendency towards higher values *(Agner 1991 (VIII))*.

The exact etiology of hyperreactive skin remains unknown. Apart from the presence of an acute eczema, other internal factors are also known to influence skin reactivity. Decreased response to croton oil in patients with severe cancer as compared to controls was reported *(Johnson et al 1971)*. Cortisol treatment may also reduce skin reactivity *(Roper & Jones 1982)*. Changes in skin reactivity to SLS during the menstrual cycle were demonstrated *(Agner et al 1991 (VI))*. The influence of a generalized infectious disease on skin reactivity – if any – may be speculated upon.

Conclusions about noninvasive measuring methods

Measuring methods are per definition objective, as compared to the subjective visual assessment of skin responses. However, if the use of the techniques is not carefully standardized with respect to existing sources of variation their objectiveness may be invalidated. Therefore it is of the utmost importance that the use of noninvasive techniques for the investigation of contact dermatitis should be optimized and standardized.

Quantification of skin response to irritants. Using the SLS patch test as a model, we found that TEWL was superior in sensitivity to laser Doppler flowmetry, colour measurement by a tri-stimulus colorimeter and ultrasound A-scan *(Agner & Serup 1990 (IV))*. High sensitivity of laser Doppler flowmetry and ultrasound was confined to certain intervals after removal of the patch test chambers, 48 and 24 h respectively *(Agner & Serup 1990 (IV))*. Colour measurement, as indicated by changes in the a*-axis, was consistently found to be the least useful method for evaluation of SLS-induced skin damage *(Agner & Serup 1990 (IV))*. These conclusions are limited to SLS-induced skin damage.

Application of noninvasive measuring methods to clinical eczema is often disturbed by heterogeneity in the clinical picture. Each method measures one single parameter contributing to the full picture of skin damage. Clinical evaluation of the skin response can incorporate more variables at a time, although essentially subjective. Noninvasive methods are found to be important tools in experimental studies, and in obtaining specific information about a single parameter, while their use for routine purposes, such as evaluation of conventional patch test reactions, may not be helpful.

For assessment of "sensitive skin". The association found between baseline TEWL and skin susceptibility to SLS indicates the possibility of detecting and following up a group at risk of developing irritant contact dermatitis. The association between "fair" and sensitive skin also indicates this possibility.

Determinants of skin sensitivity – conclusions and future aspects

Individual- as well as environment-related factors influence skin susceptibility to SLS. Information about these variables may contribute pieces to the puzzle of susceptible skin. Since irritant contact dermatitis may often show a chronic and sometimes perhaps permanent course, intervention before the development of clinical disease would be preferable.

Epidemiological studies have given some important clues to the identification of high-risk subjects. Our experimental studies have demonstrated an association between baseline TEWL, "fair" skin and skin susceptibility. Other biophysical skin properties as skin pH *(Wilhelm & Maibach 1991)* or skin hydration may also be of relevance. Prospective long-term follow-up studies are necessary to determine finally the value of noninvasive measurements for prediction of irritant contact dermatitis. These studies should include persons entering high risk professions. Results of the measurements should be evaluated together with anamnestic information of risk factors, and the importance of each factor assessed.

SUMMARY IN ENGLISH

The aim of the study was to assess the susceptibility of clinically normal skin to a standard irritant trauma under varying physiological and patophysiological conditions.

Evaluation of skin responses to patch tests with sodium lauryl sulphate (SLS) was used for assessment of skin susceptibility. The following noninvasive measuring methods were used for evaluation of the skin before and after exposure to irritants: measurement of transepidermal water loss by an evaporimeter, measurement of electrical conductance by a hydrometer, measurement of skin blood flow by laser Doppler flowmetry, measurement of skin colour by a colorimeter and measurement of skin thickness by ultrasound A-scan. The studies were carried out on healthy volunteers and patients with eczema.

In the first studies the standard irritant patch test for assessment of skin susceptibility was characterized and validated. SLS was chosen among other irritants because of its ability to penetrate and impair the skin barrier. The implications of use of different qualities of SLS was investigated. The applied noninvasive measuring methods were evaluated, and for quantification of SLS-induced skin damage measurement of TEWL was found to be the most sensitive method.

Application of the standard test on clinically normal skin under varying physiological and patophysiological conditions lead to the following main results: Seasonal variation in skin susceptibility to SLS was found, with increased susceptibility in winter, when the hydration state of the stratum corneum was also found to be decreased. A variation in skin reactivity to SLS during the menstrual cycle was demonstrated, with an increased skin response at day 1 as compared to days 9-11 in the menstrual cycle. The presence of active eczema distant from the test site increased skin susceptibility to SLS, indicating a generalized hyperreactivity of the skin.

Taking these sources of variation into account healthy volunteers and patients with hand eczema and atopic dermatitis were studied and compared. In healthy volunteers increased baseline TEWL and increased light reflection from the skin, interpreted as "fair" skin, was found to be associated with increased susceptibility to SLS. Hand eczema patients were found to have fairer and thinner skin than matched controls. Increased susceptibility to SLS was found only in patients with acute eczema. Patients with atopic dermatitis had increased baseline TEWL as well as increased skin susceptibility as compared to controls.

Skin susceptibility is thus influenced by individual- as well as environment-related factors. Knowledge of determinants of skin susceptibility may be useful for the identification of high-risk subjects for development of irritant contact dermatitis, and may help to prevent the formation of the disease.

SUMMARY IN DANISH

Formålet med afhandlingen er at belyse hudens reaktivitet/respons overfor et standardiseret irritativt traume under varierende fysiologiske og patofysiologiske omstændigheder, og herudfra at søge at identificere faktorer der disponerer for udvikling af irritativt kontakteksem.

Som irritativ standardtest er brugt epikutantestning, fortrinsvis med detergenten natriumlaurylsulfat. Såvel før som efter udsættelse for standardtesten blev huden evalueret ved hjælp af følgende noninvasive teknikker: måling af transepidermalt vandtab med et evaporimeter, måling af elektrisk konduktans med et hydrometer, måling af hudens gennemblødning med laser Doppler flowmetri, måling af hudtykkelse med ultralyd A-scan og farvereflektans-måling med et colorimeter. I de indledende studier er den anvendte standardtest evalueret, og betydningen af anvendelse af varierende kemiske kvaliteter af natriumlaurylsulfat belyst. Intra- og interindividuel variation i hudrespons, og usikkerhed på målemetoderne blev undersøgt. Måling af transepidermalt vandtab fandtes gennemgående mere sensitiv end de øvrige metoder til kvantitering af det af standardtesten fremkaldte hudrespons.

Hos raske frivillige forsøgsdeltagere påvistes hudens reaktivitet overfor standardtesten at afhænge af årstiden, med øget reaktivitet i vinterperioden. Ligeledes fandtes hos kvinder en afhængighed af menstruationscyklus, idet standardtesten udløste et kraftigere respons 1. dag end 9.-11. dag i menstruationscyklus.

Hudraske personer med høj reaktivitet (kraftigt respons) var karakteriserede ved højt basalt transepidermalt vandtab og lys hud. Ved sammenligning af ikke-involveret hud hos håndeksempatienter og hos raske fandtes signifikant lysere hud samt tyndere hud blandt eksempatienterne. En generaliseret øget hudreaktivitet hos håndeksempatienter kunne ikke påvises, men øget hudreaktivitet fandtes hos patienter med akut eksem. På klinisk normal hud hos patienter med atopisk dermatitis fandtes forhøjet basalt transepidermalt vandtab og en øget hudreaktivitet overfor standardtesten sammenlignet med hudraske personer.

Ud fra de gennemførte undersøgelser konkluderes, at hudens reaktivitet er influeret af en række faktorer relateret til det enkelte individ såvel som til det omgivende miljø. Hudens reaktivitet overfor natriumlaurylsulfat moduleres afhængigt af årstid og tidspunkt i menstruationscyklus, og tilstedeværelsen af et lokaliseret aktivt eksem et sted på kroppen øger hudens reaktivitet andre steder på kroppen. Højt basalt transepidermalt vandtab samt lys hud er associeret med en øget reaktivitet overfor natriumlaurylsulfat. Viden om faktorer med indflydelse på hudens reaktivitet overfor irritative påvirkninger, kan anvendes til forebyggelse af irritativ kontakt dermatitis.

REFERENCES

Agner T, Serup J. Skin reaction to experimental irritants assessed by polysulfide rubber replica. Contact Dermatitis 1987; 17: 205-11.

Agner T, Serup J. Contact thermography for assessment of skin damage due to experimental irritants. Acta Derm Venereol (Stockh) 1988a; 68: 192-195.

Agner T, Serup J. Comparison of two electrical methods for measurement of skin hydration. An experimental study on irritant patch test reactions. Bioengineering and the Skin 1988b; 4: 263-269.

Agner T, Serup J. Skin reactions to irritants assessed by non-invasive, bioengineering methods. Contact Dermatitis 1989; 20: 352-59. (I).

Agner T, Serup J, Handlos V, Batsberg W. Different skin irritation abilities of different qualities of sodium lauryl sulphate. Contact Dermatitis 1989; 21: 184-188. (II).

Agner T, Serup J. Seasonal variation of skin resistance to irritants. Br J Dermatol 1989; 121: 323-328. (V).

Agner T, Serup J. Quantitation of the DMSO-response: A test for sensitive skin. Clinical and Experimental Dermatology 1989; 14: 214-17.

Agner T, Serup J. Individual and instrumental variations in irritant patch-test reactions – clinical evaluation and quantification by bioengineering methods. Clinical and Experimental Dermatology 1990; 15: 29-33. (III).

Agner T, Serup J. Sodium lauryl sulphate for irritant patch testing – A dose-response study using bioengineering methods for determination of skin irritation. J Invest Dermatol 1990; 95: 543-547. (IV).

Agner T, Fullerton A, Broby-Johansen U, Batsberg W. Irritant patch testing: Penetration of sodium lauryl sulphate (SLS) into human skin. Skin Pharmacology 1990a; 3: 213-217.

Agner T, Serup J. Transepidermal water loss and air convection. Contact Dermatitis 1990b; 22: 120-121.

Agner T, Damm P, Skouby SO. Menstrual cycle and skin reactivity. J Am Acad Dermatol 1991; 24: 566-570. (VI).

Agner T. Basal transepidermal water loss, skin thickness, skin blood flow and skin colour in relation to sodium-lauryl- sulphate-induced irritation in normal skin. Contact Dermatitis 1991; 25: 108-114. (VII).

Agner T. Skin susceptibility in uninvolved skin of hand eczema patients and healthy controls. Br J Dermatol 1991; 125: 140-146. (VIII).

Agner T. Susceptibility of atopic dermatitis patients to irritant dermatitis caused by sodium lauryl sulphate. Acta Derm Venereol (Stockh) 1991; 71: 296-300. (IX).

Agner T, Serup J. Transepidermal water loss following 24 h patch testing with sodium lauryl sulphate and water. Submitted.

Agner T. An experimental study of irritant effects of urea in different vehicles. In press.

Alexander H, Miller DL. Determining skin thickness with pulsed ultrasound. J Invest Dermatol 1979; 72: 17-19.

Alexander S. Patch testing and menstruation. The Lancet 1988; i: 751.

Al-jaberi H, Marks R. Studies of the clinically uninvolved skin in patients with dermatitis. Br J Dermatol 1984; 111: 437-443.

Andersen KE, Maibach HI. Black and white human skin differences. J Am Acad Dermatol 1979; 1: 276-282.

Andersen KE, Maibach HI. Cumulative irritancy in the guinea pig from low grade irritant vehicles and the angry skin syndrome. Contact Dermatitis 1980; 6: 430-434.

Andersen KE, Benezra C, Burrows D, Camarasa J, Dooms- Goosens A, Ducombs G, Frosch P, Lachapelle J, Lahti A, Menne T, Rycroft R, Scheper R, White I, Wilkinson J. Contact Dermatitis. A review. Contact Dermatitis 1987; 16: 55-78.

Babulak SW, Rhein LD, Scala DD, Simion FA. Quantitation of erythema in a soap chamber test using the Minolta Chroma (reflectance) Meter: Comparison of instrumental results with visual assessment. J Soc Cosmet Chem 1986; 37: 475-479.

Baker H, Kligman AM. Measurement of transepidermal water loss by electrical hygrometry. Arch Derm 1967; 96: 441-452.

Baker H. The effects of dimethylsulfoxide, dimethylformamide and dimethylacetamide on the cutaneous barrier to water in human skin. J Invest Dermatol 1968; 50: 283-288.

Berardesca E, Maibach HI. Bioengineering and the patch test. Contact Dermatitis 1988a; 18: 3-9.

Berardesca E, Maibach HI. Racial differences in sodium lauryl sulphate induced cutaneous irritation: black and white. Contact Dermatitis 1988b; 18: 65-70.

Berardesca E, Maibach HI. Sodium-lauryl-sulphate-induced cutaneous irritation. Comparison of white and hispanic subjects. Contact Dermatitis 1988c; 19: 136-140.

Bettley FR, Grice K. The influence of ambient humidity on transepidermal water loss. Br J Dermatol 1967; 78: 575-581.

Björnberg A. Skin reactions to primary irritants in patients with hand eczema. 1968; Isacson, Göteborg.

Björnberg A. Skin reactions to primary irritants in men and women. Acta Derm Venereol (Stockh) 1975; 55: 191-194.

Björnberg A, Löwhagen G, Tengberg J. Relationship between intensities of skin test reactions to glass-fibres and chemical irritants. Contact Dermatitis 1979; 5: 171-174.

Blanken R, van der Valk PGM, Nater JP. Laser-Doppler flowmetry in the investigation of irritant compounds on human skin. Dermatosen 1986; 34: 5-9.

Blichmann C, Serup J. Hydration studies on scaly hand eczema. Contact Dermatitis 1987; 16: 155-159.

Blichmann C, Serup J. Assessment of skin moisture. Acta Derm Venereol (Stockh) 1988; 68: 284-290.

Brazier S, Shaw S. High-frequency ultrasound measurement of patch test reactions. Contact Dermatitis 1986; 15: 199-201.

Bruynzeel DP, van Ketel WG, Scheper RJ, von Blomberg-van der Flier BME. Delayed time course of irritation by sodium lauryl sulphate: Observations on treshold reactions. Contact Dermatits 1982; 8: 236-239.

Bruynzeel DP, van Ketel WG, von Blomberg-van der Flier M, Scheper RJ. Angry back or the excited skin syndrome. J Am Acad Dermatol 1983; 8: 392-397.

Bruynzeel DP, Maibach HI. Excited skin syndrome and the hyporeactive state: current status. In: Menné & Maibach: Exogenous dermatoses: environmental dermatitis. 1991; pp 142-150. CRC Press Inc, Florida.

Burckhardt W. Neure Untersuchungen bei die Alkali-empfindlichkeit der Haut. Dermatologica 1947; 94: 73-96.

Burckhardt W. Praktische und theoretische Bedeutung der Alkalineutralisations- und Resistenzproben. Arch Klin Exp Derm 1964; 219: 600-603.

Coenraads PJ, Bleumink E, Nater JP. Susceptibility to primary irritants. Contact Dermatitis 1975; 1: 377-381.

Coenraads PJ, Pinnagoda J. Dermatitis and water vapour loss in metal workers. Contact Dermatitis 1985; 13: 347-348.

Coenraads PJ, Lee J, Pinnagoda J. Changes in water vapour loss from the skin of metal industry workers monitored during exposure to oils. Scand J Work Environ Health 1986; 12: 494-498.

Cua AB, Wilhelm KP, Maibach HI. Cutaneous sodium lauryl sulphate irritation potential: age and regional variability. Contact Dermatitis 1990; 23: 276. Abstract.

Dahl MV, Trancik RJ. Sodium lauryl sulphate irritant patch tests: Degree of inflammation at various times. Contact Dermatitis 1977; 3: 263-266.

Dahl MV, Pass F, Trancik RJ. Sodium lauryl sulfate irritant patch tests. II Variation of test responses among subjects and comparison to variations of allergic responses elicited by Toxicodendron extract. J Am Acad Dermatol 1984; 11: 474-477.

Dahl MV, Roering MJ. Sodium lauryl sulphate irritant patch tests. III. Evaporation of aquous vehicle influences inflammatory response. J Am Acad Dermatol 1984; 11: 477-479.

de Boer EM, Bezemer PD, Bruynzeel DP. A standard method for repeated recording of skin blood flow using laser Doppler flowmetry. Dermatosen 1989; 37: 58-62.

de Boer EM, Scholten JPM, van Ketel WG, Bruynzeel DP. The irritancy of metal working fluids: a laser Doppler flowmeter study. Contact Dermatitis 1990; 22: 86-94.

Diffey BL, Oliver RJ, Farr PM. A portable instrument for quantifying erythema induced by ultraviolet radiation. Br J Dermatol 1984; 111: 663-672.

Dupuis D, Rougier A, Lotte C, Wilson D, Maibach HI. In vivo relationship between percutaneous absorption and transepidermal water loss according to anatomic site in man. J Soc Cosmet Chem 1986; 37: 351-357.

Edwards C, Payne PA. Ultrasonic velocities in skin components. Abstract presented in International Society of Bioengineering of the Skin, 9 November 1984; Liege, Belgium.

Elias PM, Cooper ER, Korc A, Brown BE. Percutaneous transport in relation to stratum corneum structure and lipid composition. J Invest Dermatol 1981; 76: 297-301.

Elsner P, Wilhelm D, Maibach HI. Irritant dermatitis and aging. Contact Dermatitis 1990; 23: 275. Abstract.

Escoffier C, Querleux B, De Rigal J, Leveque JL. In vitro study of the velocity of ultrasound in the skin. Bioengineering and the skin 1986; 2: 87-94.

Farr PM, Diffey BL. Quantitative studies on cutaneous erythema induced by ultraviolet radiation. Br J Dermatol 1984; 111: 673-682.

Feldmann RJ, Maibach HI. Regional variation in percutaneous penetration of 14C cortisol in man. J Invest Dermatol 1967; 48: 181-183.

Fischer T, Maibach H. Finn chamber patch test technique. Contact Dermatitis 1984; 11: 137-140.

Flannigan SA, Smith RE, McGovern JP. Intraregional variation between contact irritant patch test sites. Contact Dermatitis 1984; 10: 123-124.

Freeman S, Maibach HI. Study of irritant contact dermatitis produced by repeat patch testing with sodium lauryl sulphate and assessed by visual methods, transepidermal water loss and laser Doppler velocimetry. J Am Acad Dermatol 1988; 19: 496-502.

Freeman RG, Cockerell EG, Armstrong J, Knox JM. Sunlight as a factor influencing the thickness of the epidermis. J Invest Dermatol 1962; 39: 295-298.

Frosch PJ, Kligman AM. The chamber-scarification test for irritancy. Contact Dermatitis 1976; 2: 314-324.

Frosch PJ, Kligman AM. Rapid blister formation in human skin with ammonium hydroxide. Br J Dermatol 1977; 96: 461-473.

Frosch PJ, Kligman AM. The soap chamber test. J Am Acad Dermatol 1979a; 1: 35-41.

Frosch PJ, Kligman AM. The Duhring chamber. Contact Dermatitis 1979b; 5: 73-81.

Frosch PJ, Duncan S, Kligman AM. Cutaneous biometrics I. The response of human skin to dimethyl sulphoxide. Br J Dermatol 1980; 102: 263-274.

Frosch PJ, Kligman AM. Recognition of chemically vulnerable and delicate skin. In: Frost P & Horwitz S. Principles of cosmetics for the dermatologist. 1982; pp 287-296, the C.V.Mosby Company, St. Louis.

Frosch PJ, Wissing C. Cutaneous sensitivity to ultraviolett light and chemical irritants. Arch Dermatol Res 1982; 272: 269-278.

Frosch PJ. Hautirritation und empfindliche Haut. 1985; Grosse Verlag, Berlin.

Grice K, Sattar H, Sharratt M, Baker H. Skin temperature and transepidermal water loss. J Invest Dermatol 1971; 57: 108-110.

Goh CL, Chia SE. Skin irritability to sodium lauryl sulphate as measured by skin vapour loss by sex and race. Clinical and Experimental Dermatology 1988; 13: 16-19.

Gollhausen R, Kligman AM. Effects of pressure on contact dermatitis. American Journal of Industrial Medicine 1985; 8: 323-328.

Halter K. Zur pathogenese des Ekzems. Arch Derm U Syph 1941; 181: 593-719.

Hanifin JM, Rajka G. Diagnostic features of atopic dermatitis. Acta Derm Venereol (Stockh) 1980; suppl 92, 44-47.

Hannuksela M, Pirilä V, Salo OP. Skin reactions to propylene glycol. Contact Dermatitis 1975; 1: 112-116.

Hannuksela M, Salo H. The repeated open application test (ROAT). Contact Dermatitis 1986; 14: 221-227.

Hattingh J. The influence of skin temperature, environmental temperature and relative humidity on transepidermal water loss. Acta Derm Venereol (Stockh) 1972; 52: 438-440.

Hjorth N. Seasonal variations in contact dermatitis. Acta Derm Venereol (Stockh) 1967; 47: 409-418.

Holst R, Möller H. One hundred twin pairs patch tested with primary irritants. Br J Dermatol 1975; 93: 145-149.

Jemec GBE, Agner T, Serup J. Transonychial water loss: relation to sex, age and nail-plate thickness. Br J Dermatol 1989; 121: 443-446.

Johnson MW, Maibach HI, Salmon SE. Skin reactivity in patients with cancer. New Engl J Med 1971; 284: 1255-1256.

Kemmet D. Premenstrual exacerbation of atopic dermatitis. Br J Dermatol 1989; 120: 715.

Kligman AM. Topical pharmacology and toxicology of dimethyl sulfoxide. JAMA 1965; 193: 140-148.

Kligman AM, Wooding WM. A method for the measurement and evaluation of irritants on human skin. J Invest Dermatol 1967; 49: 78-94.

Kligman AM. Assessment of mild irritants in humans. In: Drill and Lazar. Current concepts in cutaneous toxicity. 1980; pp 69-94, Academic press, New York.

Kligman AM, Gollhausen R. The angry back: a new concept or old confusion? Br J Dermatol 1986; 115: suppl 31: 93-100.

Lammintausta K, Maibach HI, Wilson D. Human cutaneous irritation: induced hyporeactivity. Contact Dermatitis 1987a; 17: 193-198.

Lammintausta K, Maibach HI, Wilson D. Irritant reactivity in males and females. Contact Dermatitis 1987b; 17: 276-280.

Lammintausta K, Maibach HI, Wilson D. Susceptibility to cumulative and acute irritant dermatitis. Contact Dermatitis 1988; 19: 84-90.

Lanman BM, Elvers WB, Howard CS. The role of human patch testing in a product development program. In: Proceedings of the joint conference on cosmetic science, the Toilet Goods Association, Washington DC 1968; 135-145.

Larmi E, Lahti A, Hannuksela M. Effect of ultraviolet B on nonimmunologic contact reactions induced by dimethyl sulfoxide, phenol and sodium lauryl sulphate. Photodermatology 1989; 6: 258-262.

Leveque JL, de Rigal J. Impedance methods for studying skin moisturization. J Soc Cosmet Chem 1983; 34: 419-428.

Magnusson B, Hersle K. Patch test methods. I. A comparative study of six different types of patch tests. Acta Derm Venereol (Stockh) 1965a; 45: 123-128.

Magnusson B, Hersle K. Patch test methods. II. Regional variation of patch test responses. Acta Derm Venereol (Stockh) 1965b; 45: 257-261.

Malten KE, den Arend JA, Wiggers RE. Delayed irritation: Hexanediol diacrylate and butanediol diacrylate. Contact Dermatitis 1979; 5: 178-184.

Malten KE. Thoughts on irritant contact dermatitis. Contact Dermatitis 1981; 7: 238-247.

Mathias CGT, Wilson DM, Maibach HI. Transepidermal water loss as a function of skin surface temperature. J Invest Dermatol 1981; 77: 219-220.

Meding B, Swanbeck G. Epidemiology of different types of hand eczema in an industrial city. Acta Derm Venereol (Stockh) 1989; 69: 227-233.

Meding B, Swanbeck G. Occupational hand eczema in an industrial city. Contact Dermatitis 1990; 22: 13-23.

Mitchell JC. Multiple concomitant positive patch test reactions. Contact Dermatitis 1977; 3: 315-320.

Murahata R, Crove DM, Roheim JR. The use of transepidermal water loss to measure and predict the irritation response to surfactants. Int J Cosmetic Science 1986; 8: 225-231.

Mustakallio KK. Irritation and staining by dithranol (anthralin) and related compounds: I. Estimation with chamber testing and contact thermography. Acta Derm Venereol (Stockh) 1979; 59, Suppl. 85: 125-132.

Myers MJ, Butler LD, Petersen BH. Estradiol-induced alteration in the immune system. Suppression of cellular immunity in the rat is not the result of direct estrogenic action. Immunopharmacology 1986; 11: 47-55.

Nilsson GE, Otto U, Wahlberg JE. Assessment of skin irritancy in man by laser Doppler flowmetry. Contact Dermatitis 1982; 8: 401-406.

Nilsson GE. Perimed's LDV flowmeter. In Shepherd & Öberg: Laser Doppler blood flowmetry. 1990; pp 57-72. Kluwer Academic publishers, Massachusetts.

Nilsson E, Bäck O. The importance of anamnestic information of atopy, metal dermatitis and earlier hand eczema for the development of hand dermatitis in women in wet hospital work. Acta Derm Venereol (Stockh) 1986; 66: 45-50.

Novak E, Francom SF. Inflammatory response to SLS in aqueous solutions applied to the skin of normal human volunteers. Contact Dermatitis 1984; 10: 101-104.

Pirilä V. Chamber test versus patch test for epicutaneous testing. Contact Dermatitis 1975; 1: 48-52.

Pinnagoda J, Tupker RA, Coenraads PJ, Nater JP. Prediction of susceptibility to an irritant response by transepidermal water loss. Contact Dermatitis 1989a; 20: 341-346.

Pinnagoda J, Tupker RA, Smit JA, Coenraads PJ, Nater JP. The intra- and inter-individual variability and reliability of transepidermal water loss measurements. Contact Dermatitis 1989b; 21: 255-259.

Pinnagoda J, Tupker RA, Coenraads PJ, Nater JP. Transepidermal water loss with and without sweat gland inactivation. Contact Dermatitis 1989c; 21: 16-22.

Pinnagoda J, Tupker RA, Agner T, Serup J. Guidelines for transepidermal water loss (TEWL) measurement. Contact Dermatitis 1990; 22: 164-178.

Queille-Roussel C, Poncet M, Schaefer H. Quantification of skin colour changes induced by topical corticosteriods on normal skin using the Minolta chroma meter. Abstract presented at the 8th international symposium

on bioengineering and the skin, Stresa, Italy, June 1990a.

Queille-Roussel C, Duteil L, Padilla J, Poncet M, Czernielewski J. Objective assessment of topical anti-inflammatory drug activity on experimentally induced nickel contact dermatitis: comparison between visual scoring, colorimetry, laser Doppler velocimetry and transepidermal water loss. Skin Pharmacology 1990b; 3: 248-255.

Robertson AR. The CIE 1976 color difference formulas. Color Research and Application 1977; 2: 7 – 11.

Roper S, Jones HE. A new look at conditioned hyperirritability. J Am Acad Dermatol 1982; 7: 643-650.

Rycroft RJG, Smith WDL. Low humidity occupational dermatoses. Contact Dermatitis 1980; 6: 488-492.

Rystedt I. Factors influencing the occurence of hand eczema in adults with a history of atopic dermatitis in childhood. Contact Dermatitis 1985a; 12: 185-191.

Rystedt I. Work-related hand eczema in atopics. Contact Dermatitis 1985b; 12: 164-171.

Rystedt I. Atopic background in patients with occupational hand eczema. Contact Dermatitis 1985c; 12: 247-254.

Schulz D, Korting GW. Zur weiteren Kenntnis der Alkaliresistenz-Probe. Dermatosen 1987; 35: 91-94.

Seitz, JC, Whitmore CG. Measurement of erythema and tanning responses in human skin using a tri-stimulus colorimeter. Dermatologica 1988; 177: 70-75.

Serup J, Staberg B, Klemp P. Quantification of cutaneous oedema in patch test reactions by measurement of skin thickness with high-frequency pulsed ultrasound. Contact Dermatitis 1984; 10: 88-93.

Serup J, Staberg B. Differentiation of allergic and irritant reactions by transepidermal water loss. Contact Dermatitis 1987a; 16: 129-132.

Serup J, Staberg B. Ultrasound for assessment of allergic and irritant patch test reactions. Contact Dermatitis 1987b; 17: 80-84.

Serup J, Agner T. Colorimetric quantification of erythema – a comparison of two colorimeters (Lange Micro Color and Minolta Chroma Meter CR-200) with a clinical scoring scheme and laser Doppler flowmetry. Clinical and Experimental Dermatology 1990; 15: 267-272.

Serup, J. Ten years experience with high frequency ultrasound examination of the skin: Development and refinement of technique and equipments. Ed. P Altmeyer, Bochum. Karger, in press.

Shahidullah M, Raffle EJ, Rimmer AR, Frain-Bell W. Transepidermal water loss in patients with dermatitis. Br J Dermatol 1969; 81: 722-730.

Shepherd AP. History on LDV flowmetry. In Shepherd & Öberg: Laser Doppler blood flowmetry. 1990; pp 1-16, Kluwer Academic publishers, Massachusetts.

Staberg B, Serup J. Allergic and irritant skin reactions evaluated by laser Doppler flowmetry Contact Dermatitis 1988; 18: 40-45.

Stillman MA, Maibach HI, Shalita AR. Relative irritancy of free fatty acids of different chain length. Contact Dermatitis 1975; 1: 65-69.

Spencer T, Linamen CE, Akers WA, Jones HE. Temperature dependence of water content of the stratum corneum. Br J Dermatol 1975; 93: 159-164.

Tagami H, Ohi M, Iwatsuki K, Kanamaru Y, Yamada M, Ichijo B. Evaluation of the skin surface hydration in vivo by electrical measurement. J Invest Dermatol 1980; 75: 500-507.

Tagami H. Impedance measurement for evaluation of the hydration state of the skin surface. In: Leveque J: Cutaneous investigation in health and disease, Marcel Dekker Inc 1990; 79-111.

Thiele FAJ, Malten KE. Evaluation of skin damage. Skin resistance measurements with alternating current (impedance measurements). Br J Dermatol 1973; 89: 373-382.

Tupker RA, Pinnagoda J, Coenraads PJ, Nater JP. The influence of repeated exposure to surfactants on the human skin as determined by transepidermal water loss and visual scoring. Contact Dermatitis 1989a; 20: 108-114.

Tupker RA, Coenraads PJ, Pinnagoda J, Nater JP. Baseline transepidermal water loss (TEWL) as a prediction of susceptibility to sodium lauryl sulphate. Contact Dermatitis 1989b; 20: 265-269.

Tupker RA, Pinnagoda J, Nater JP. The transient and cumulative effect of sodium lauryl sulphate on the epidermal barrier assessed by transepidermal water loss: inter-individual variation. Acta Derm Venereol (Stockh) 1990a; 70: 1-5.

Tupker RA, Pinnagoda J, Coenraads PJ, Nater JP. Susceptibility to irritants: role of barrier function, skin dryness and history of atopic dermatitis. Br J Dermatol 1990b; 123: 199-205.

van der Valk PGM, Nater JP, Bleumink E. Skin irritancy of surfactants as assessed by water vapour loss measurements. J Invest Dermatol 1984; 82: 291-293.

van der Valk PGM, Vries MHK, Nater JP, Bleumink E, de Jong MCJM. Eczematous (irritant and allergic) reactions of the skin and barrier function as determined by water vapour loss. Clinical and Experimental Dermatology 1985a; 10: 185-193.

van der Valk PGM, Nater JP, Bleumink E. Vulnerability of the skin to surfactants in different groups of eczema patients and controls as measured by water vapour loss. Clinical and Experimental Dermatology 1985b; 10: 98-103.

Wahlberg JE, Maibach HI. Nonanoic acid irritation – A positive control at routine patch testing? Contact Dermatitis 1980; 6: 128-130.

Wahlberg JE, Nilsson G. Skin irritancy from propylene glycol. Acta Derm Venereol (Stockh) 1984; 64: 286-290.

Wahlberg JE, Wrangsjö K, Hietasalo A. Skin irritancy from nonanoic acid. Contact Dermatitis 1985; 13: 266-269.

Wahlberg JE. Evaluation of test reactions. Acta Derm Venereol (Stockh) 1988; 68, suppl 135: 12-15.

Wall LM, Gebauer KA. A follow-up study of occupational skin disease in Western Australia. Contact Dermatitis 1991; 24: 241-243.

Warshaw TG, Hermann F. Studies on skin reactions to propylenglycol. J Invest Dermatol 1952; 19: 423-430.

Weigand DA, Haygood C, Gaylor JR. Cell layers and density of negro and caucasian stratum corneum. J Invest Dermatol 1974; 62: 563-568.

Westerhof W, Estevez-Uscanga O, Meens J, Kammeyer A, Durocq M, Cario I. The relationship between constitutional skin colour and photosensitivity estimated from UV-induced erythema and pigmentation dose-response curve. J Invest Dermatol 1990; 94: 812-816.

Werner Y, Lindberg M. Transepidermal water loss in dry and clinically normal skin in patients with atopic dermatitis. Acta Derm Venereol (Stockh) 1985; 65: 102-105.

Werner Y. The water content of the stratum corneum in patients with atopic dermatitis. Acta Derm Venereol (Stockh) 1986; 66: 281-284.

Wilhelm KP, Surber C, Maibach HI. Quantification of sodium lauryl sulphate dermatitis in man: Comparison of four techniques:skin color reflectance, transepidermal water loss, laser Doppler flow measurement and visual scores. Arch Dermatol Res 1989; 281: 293-295.

Wilhelm KP, Maibach HI. Susceptibility to irritant dermatitis induced by sodium lauryl sulphate. J Am Acad Dermatol 1990; 23: 122-124.

Wilhelm KP, Maibach HI. Susceptibility to sodium lauryl sulfate-induced irritant dermatitis: relation to biophysical skin properties. In: Menné & Maibach: Exogenous dermatoses: environmental dermatitis. 1991; pp 236-244. CRC Press Inc, Florida.

Willis CM, Stephens JM, Wilkinson JD. Assessment of erythema in irritant contact dermatitis. Comparison between visual scoring and laser Doppler flowmetry. Contact Dermatitis 1988a; 18: 138-142.

Willis CM, Stephens CJM, Wilkinson JD. Experimentally-induced irritant contact dermatitis. Contact Dermatitis 1988b; 18: 20-24.

Willis CM, Stephens CJM, Wilkinson JD. Epidermal damage induced by irritants in man: A light and electron microscopy study. J Invest Dermatol 1989; 93: 695-699.

Wilson D, Berardesca E, Maibach HI. In vitro transepidermal water loss: differences between black and white human skin. Br J Dermatol 1988; 119: 647-652.