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Efficacy of Barrier Creams

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INTRODUCTION

The concept of barrier creams (BC) has been around since the early twentieth century. In practice, their utilization remains the subject of a lively debate; some suggest that inappropriate BC application may induce additional irritation rather than benefit (1–5).

To evaluate BC efficacy, in vivo and in vitro methods have been developed. In particular, recent bioengineering techniques provide more accurate quantitative data than has traditionally been supplied by clinical studies dependent on visual scoring.

We review the investigative details of the pertinent scientific literature and summarize methodology and efficacy of BC.

BARRIER CREAMS

BC are designed to prevent or reduce the penetration and absorption of various hazardous materials into skin, preventing skin lesions and/or other toxic effects from dermal exposure (1,2,4–7). Alternate terms for BC include ‘‘skin protective creams (SPCs),’’ ‘‘protective creams (PCs),’’ ‘‘protective ointments,’’ ‘‘invisible glove,’’ and ‘‘barrier,’’ ‘‘protective,’’ ‘‘prework’’ or ‘‘after-work’’ creams, lotions, emollients, and/or gels (7–9). Frosch et al. (4) suggest that the term ‘‘skin protective creams’’ seems more appropriate because most do not provide a barrier comparable to the stratum corneum.

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Table 1 Influence of Indulona and Ivosin on the Percutaneous Absorption of 0.017 M Chromate Solution $k \times 10^5/\text{min}^{-1}$[a]

Series no.	Barrier cream	Volume applied mL/cm^2	Interval[b] min	<3.4 n	3.4–6.6 n	6.7–10.1 n	10.2–13.5 n	13.6– n	Mean relative absorption $k \times 10^5$/min^{-1} 10 exp.	χ^2 (c), analysis of variance (v) compared with series 1
1	No barrier cream			—	4	4	—	2	8.8 ± 1.4[c]	—
2	Indulona	0.05	1	3	2	4	—	1	(5.7–6.7)[d]	$0.05 < p < 0.10$ (c)
3	Indulona	0.10	1	—	3	4	1	2	9.0 ± 1.2[c]	$p > 0.2$ (v)
4	Indulona	0.10	15	4	1	3	2	—	(5.5–6.8)[d]	$0.02 < p < 0.05$ (c)
5	Indulona	0.15	1	—	5	—	5	—	8.2 ± 0.1[c]	$p < 0.2$ (v)
6	Ivosin	0.025	1	2	2	3	3	—	(6.7–7.3)[d]	$0.10 < p < 0.20$ (c)
7	Ivosin	0.05	1	7	1	2	—	—	(2.1–4.5)[d]	$0.001 < p < 0.01$ (c)
8	Ivosin	0.10	1	8	—	2	—	—	(1.8–4.5)[d]	$p < 0.001$ (c)
9	Ivosin	0.10	15	5	5	—	—	—	(2.5–4.2)[d]	$0.001 < p < 0.01$ (c)
10	Ivosin	0.15	1	5	3	2	—	—	(3.2–4.7)[d]	$0.001 < p < 0.01$ (c)

[a] Modified from Ref. 13.
[b] Interval between application of barrier cream and test substance.
[c] Standard error.
[d] See text.

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METHODOLOGY AND EFFICACY OF BARRIER CREAMS

In 1940, Schwartz et al. (10) introduced an in vivo method to evaluate the efficacy of a vanishing cream against poison ivy extract utilizing visual erythema on human skin. The test cream was an effective prophylaxis against poison ivy dermatitis as compared to unprotected skin.

Sadler et al. (11) performed qualitative tests to evaluate the efficacy of barrier creams. One method used the fluorescence of a dyestuff and eosin as a measure of penetration; another measured the rates of penetration of water through barrier creams. These methods are rapid and simple, but provide only qualitative estimates. They introduced an apparatus for measuring the permeability of films of barrier creams.

Wahlberg (12,13) employed an isotope technique disappearance measurement for documenting the inhibiting effect of barrier creams on chromate (^{51}Cr) percutaneous absorption in guinea pigs (Table 1 and Fig. 1) (13). In this series 2,4 Indulona® (which contains Na_2H_2EDTA, $CaNa_2EDTA$ and acidum ascorbicum as active ingredients) and 6, 10 Ivosin® (which contains the hydrochloride of a copolymerizate of *p*-divinyl benzol and *p*-m-dimethylaminomethyl-

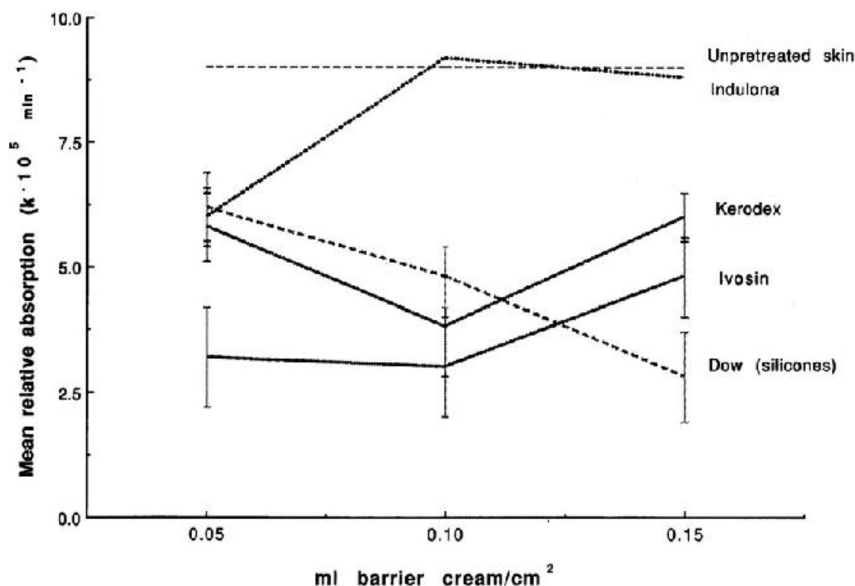

Figure 1 The effect of barrier creams on the percutaneous absorption of a 0.017 *M* aqueous solution of chromate. Three different volumes per unit area (mL/cm^2) were studied. (Modified from Ref. 13.)

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styrol) absorption decreased as a result of pretreatment. In some cases, the disappearance technique was not sufficiently sensitive to permit quantitative determination. The disappearance measurements distinguished between different barrier creams, volumes per unit area, and intervals between application of cream and chromate (13).

Langford (14) introduced in vitro studies to determine the efficacy of the formulated FC resin complex to prevent solvent penetration through treated filter paper, solvent repellency on treated pigskin, and penetration of radio-tagged sodium lauryl sulfate through treated hairless mouse skin. The penetration rate of

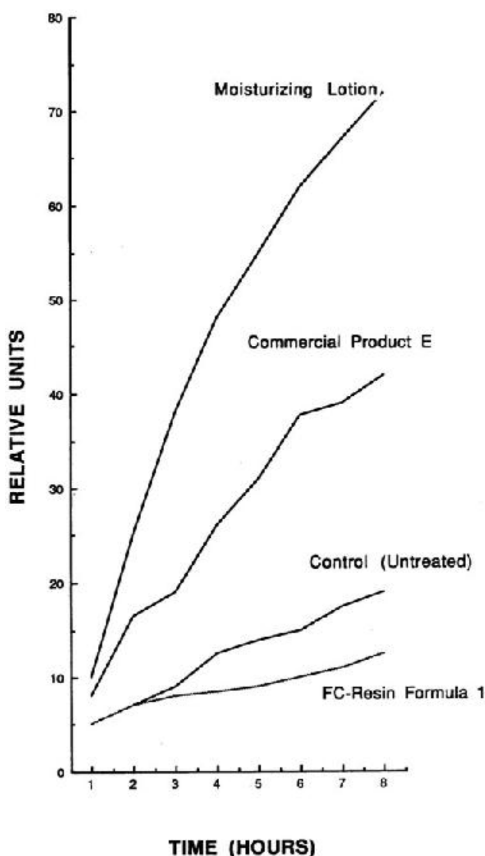

Figure 2 Penetration rate of radio-tagged sodium lauryl sulfate/ETOH through hairless mouse skin. (Modified from Ref. 14.)

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radio-tagged sodium lauryl sulfate/ETOH through hairless mouse skin is shown in Figure 2.

Reiner et al. (15) examined the protective effect of ointments both on guinea pig skin in vitro and on guinea pigs in vivo. The permeation of ''toxic agent 4''® through unprotected and protected skin within 10 h is plotted in Figure 3 as a function of time. The permeation values were determined radiologically and enzymatically. Permeation of ''toxic agent 4''® was markedly reduced by polyethylene glycol ointment base and ointments containing active substance. In in vivo experiments on guinea pigs, mortality was greater after applying the toxic

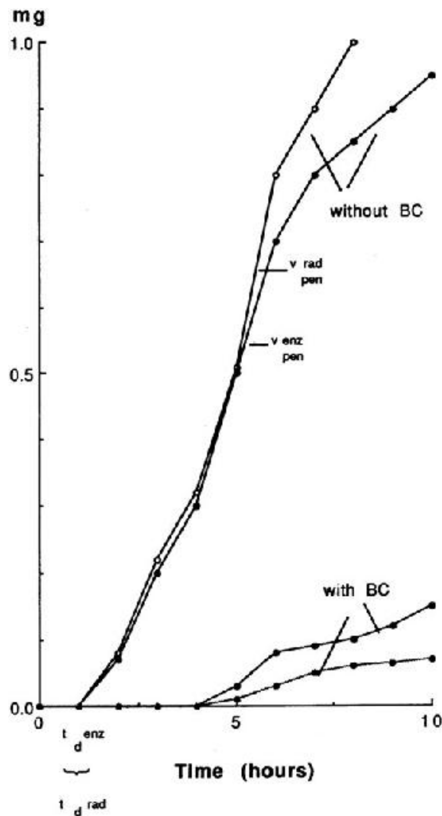

Figure 3 Permeation of the ''toxic agent 4''® through unprotected and protected skin as a function of time. ❍ = Rad data; ● = enzyme data; BC = reactive PEG ointment such as S4–S8. (Modified Ref. 15.)

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agent to unprotected skin. All formulations with nucleophilic substances markedly reduced the mortality rate.

Lachapelle et al. (16–19) utilized a guinea pig model to evaluate the protective value of barrier creams and/or gels by laser Doppler flowmetry and histological assessment. In addition, the blood concentration of *n*-hexane of the control group and the gel-pretreated group was determined. Figure 4 shows partial results (19), which correlated invasive (blood levels) and noninvasive techniques.

Loden (7) evaluated the effect of barrier creams on the absorption of (^{3}H)-water (^{14}C)-benzene, and (^{14}C)-formaldehyde into excised human skin. The control and the barrier-cream-treated skin was exposed to the test substance for 0.5 h,

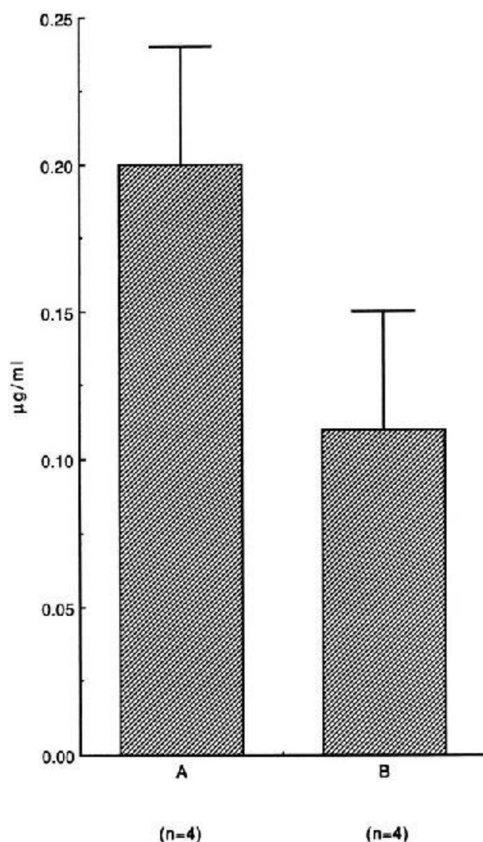

Figure 4 Mean ± SD (µg/mL) of blood levels of *n*-hexane in two groups of guinea pigs exposed for 30 min. (A) control group; (B) gel-pretreated group ($p < 0.05$). (Modified from Ref. 19.)

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whereupon absorption was determined. The experimental cream ‘‘water barrier’’ reduced the absorption of water and benzene but not formaldehyde. Kerodex 71® cream slightly reduced benzene and formaldehyde absorption. Petrogard® and ‘‘Solvent Barrier’’® did not affect the absorption of any of the substances studied (Fig. 5). One advantage of the method is the use of human skin. The effects of the barrier cream on the skin and the test substance mimic the in vivo situation. Another advantage is that the method is quantitative.

Frosch et al. (2–4,20,21) developed the repetitive irritation test (RIT) in the guinea pig and in humans to evaluate the efficacy of BC using a series of bioengineering techniques. The pretreated and untreated test skin (guinea pig or humans) was exposed daily to the irritants for 2 weeks. The resulting irritation was scored on a clinical scale and assessed by biophysical technique parameters. Some test creams suppressed irritation with all test parameters; some failed to show any effect; and some exacerbated the condition (Fig. 6) (4).

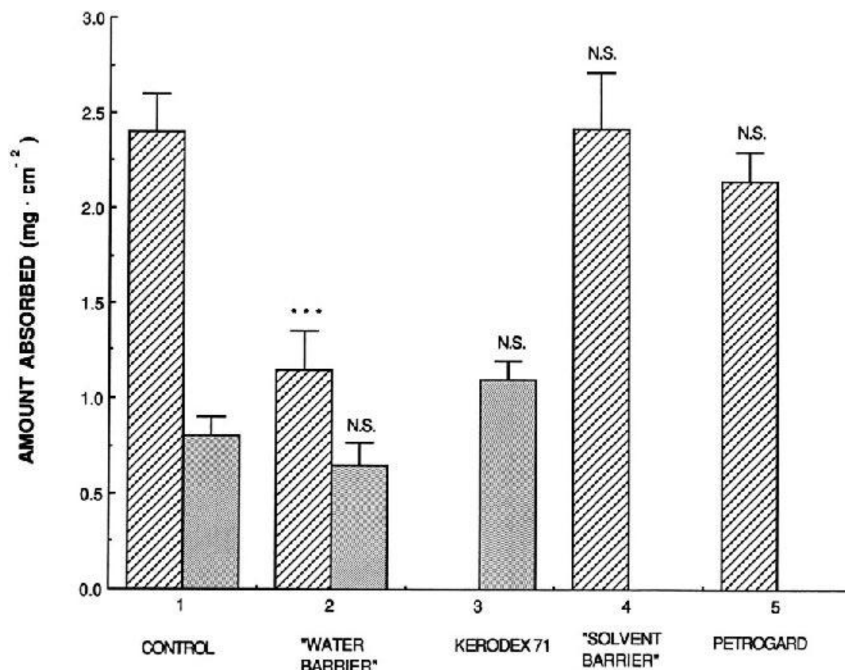

Figure 5 The amount of water absorbed into control skin and skin treated with barrier creams during 0.5 h of exposure. Skin from two donors was used (hatched and dotted columns). Values are means ± SE of the number of experiments within parenthesis. $***p < 0.001$; n.s., not significantly different from control. (Modified from Ref. 7.)

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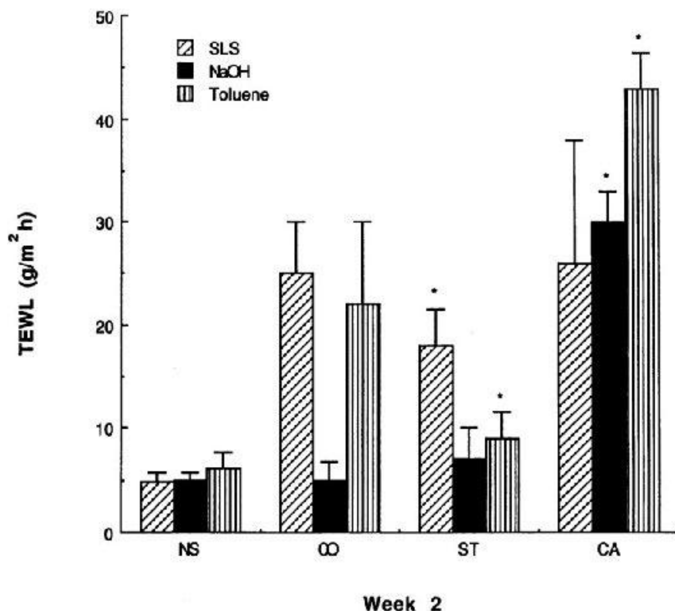

Figure 6 The effect of barrier creams: Stokoderm Salbe (ST) and Contra Alkali Creme (CA) in the guinea pig model after 2 weeks of treatment with three irritants (sodium lauryl sulfate, SLS; sodium hydroxide, NaOH; toluene). CO, control animals; NS, normal skin (untreated). Shown are the data of the transepidermal water loss (TEWL). Significant differences from control animals and barrier-cream-treated animals are indicated by an asterisk (*) ($p < 0.05$). (Modified from Ref. 4.)

Treffel et al. (22) measured the effectiveness of barrier creams on human skin in vitro against three dyes (eosin, methylviolet, and oil red O) with varying *n*-octanol/water partition coefficients (0.19, 29.8, and 165, respectively). Barrier cream efficacy was assayed by measurements of the dyes in the epidermis of protected skin samples 30 min after application. Penetration depths of the dyes into the stratum corneum are shown in Figure 7. The ΔTTC (%) related to the number of the cellophane tape strips made from the skin sample controls. The dyes were present in high amounts in the superficial layers of the stratum corneum; ΔTTC due to eosin in the first strip was lower than that obtained with both the other dyes (not statistically significant). Oil red O penetrated in greater amounts into the deeper stratum corneum. The amount of the three dyes at the bottom of the stratum corneum remains, however, low. The efficacy of barrier creams against the three dyes in several cases showed data contrary to manufac-

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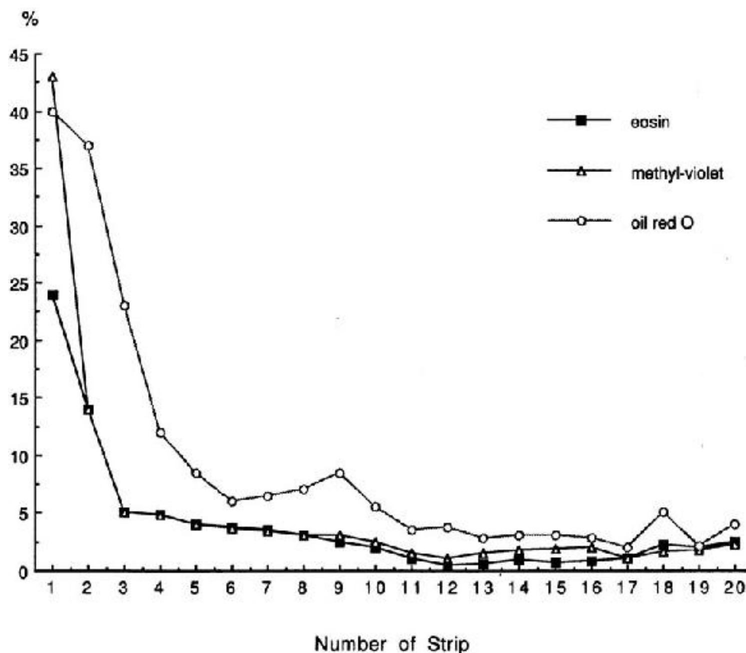

Figure 7 Δ total color change measurement in the stratum corneum expressed in percentage. (Modified from Ref. 22.)

turers' information. There was no correlation between the galenic parameters of the assayed products and the protection level, indicating that neither the water content nor the consistency of the formulations influenced the protection effectiveness.

Fullerton and Menne (23) evaluated that the protective effect of ethylenediaminetetraacetate (EDTA) barrier gels against nickel contact allergy using in vitro and in vivo methods. Thirty milligrams of barrier gel were applied on the epidermal side of the skin in vitro and a nickel disk was applied above the gel. Twenty-four hours after application, the nickel disk was removed and the epidermis separated from the dermis. Nickel content in epidermis and dermis was quantified by adsorption differential pulse voltammetry (ADPV). The distributions of nickel in the epidermis and the dermis after 24 h of occluded application of the two nickel disks made from alloy A and alloy B are in Figure 8. The amount of nickel in the epidermal skin layer after application of the barrier gels was significantly reduced compared to the untreated control.

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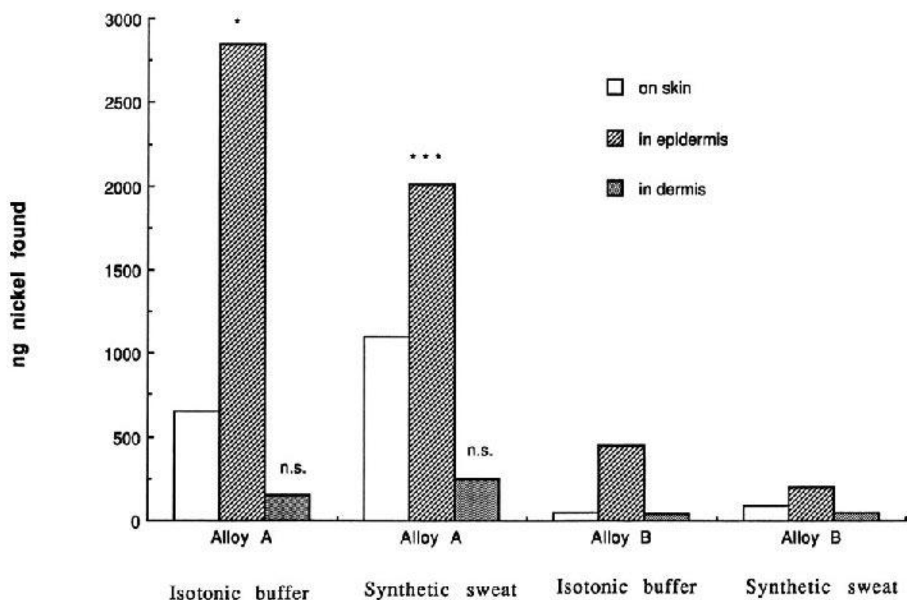

Figure 8 Distribution of nickel in epidermis and dermis after 24 h occluded application of two nickel disks made from alloy A and alloy B. Recipient mediums were isotonic phosphate buffer and synthetic sweat (skin from donor A). Statistics: 2-sample t test comparing mean skin compartment distribution of nickel after application of alloy B and alloy A. Comparison for isotonic phosphate buffer and synthetic sweat as recipient medium, respectively. n.s., no significance; $*p < 0.05$; $***p < 0.001$. (Modified from Fullerton Ref. 23.)

In vivo patch testing of nickel-sensitive patients was performed using nickel disks made of metal alloy A and Carbopol® barrier gel systems with and without added EDTA (gel type A and B). Test preparations and nickel disks were removed 1 day postapplication and the sites evaluated. Reduction in positive test reactions was highly significant.

Zhai (5) developed an in vivo method in human skin to measure the effectiveness of skin protective creams against dye indicator solutions: methylene blue in water and oil red O in ethanol, which are representative of model hydrophilic and lipophilic compounds. Solutions of 5% methylene blue and 5% oil red O were applied to untreated and protective cream pretreated skin with the aid of aluminum occlusive chambers, for 0 h and 4 h. At the end of the application time, the materials were removed, and consecutive skin surface biopsies (SSB) were obtained. The amount of dye penetrating into each strip was determined

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by colorimetry. Two creams exhibited effectiveness, but one cream enhanced a cumulative amount of dye (Fig. 9).

Zhai et al. (24) introduced a facile approach to screening protectants in vivo in human subjects. Two acute irritants and one allergen were selected: sodium lauryl sulfate (SLS) represented a household irritant that can produce contact dermatitis; the combination of ammonium hydroxide (NH^4OH) and urea was used to simulate diaper dermatitis; and Rhus was used to evaluate the effect of model protective materials. Test materials were spread over onto test area, massaged, allowed to dry for 30 min, and reapplied with another 30-min drying period. The model irritants and allergen were applied with an occlusive patch for 24 h. Inflammation was scored with an expanded 10-point scale at 72 h postapplication. Most test materials statistically suppressed the SLS irritation and Rhus allergic reaction rather than NH^4OH and urea-induced irritation (Fig. 10).

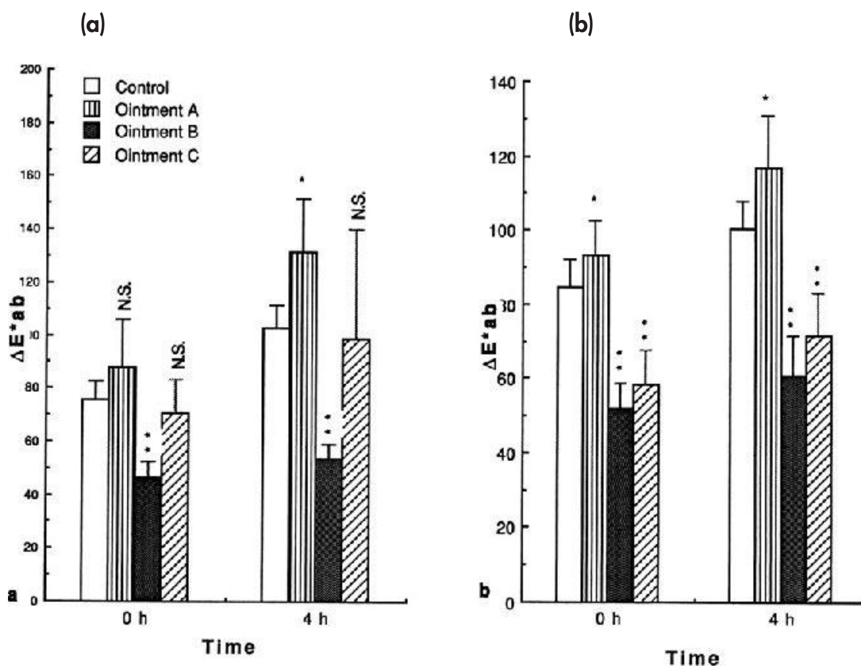

Figure 9 The amount of methylene blue and oil red O absorbed into control skin and skin treated with barrier creams: (a) methylene blue; (b) oil red O. Results expressed as the means ± s.d. of ΔE^*ab. Statistical differences in comparison to the control indicated by an asterisk (*) ($p < 0.05$) to **($p < 0.01$); n.s., not significant. (Modified from Ref. 5.)

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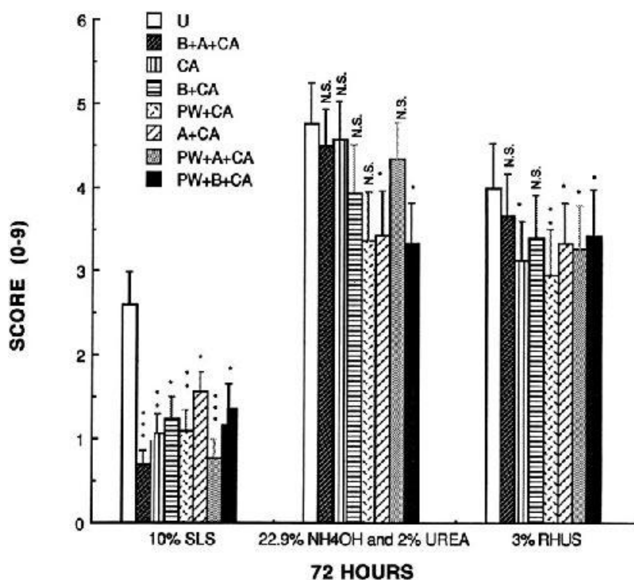

Figure 10 Efficacy of protective materials against skin irritation following treatment by two acute irritants (10% SLS, 22.9% NH^4OH and 2% urea) and 1 allergen (3% Rhus). Results expressed as the means ± SE. Statistical differences in comparison with untreated skin site. $*p < 0.05$; $**p < 0.01$; $***p < 0.001$; n.s., not significant. (Modified from Ref. 24.)

Zhai et al. (25) utilized an in vitro diffusion system to measure the protective effectiveness of quaternium-18 bentonite (Q18B) gels to prevent 1% concentration of [^{35}S]-sodium lauryl sulfate penetration by human cadaver skin. The accumulated amount of [^{35}S]-SLS in receptor-cell fluid was counted to evaluate the efficacy of the Q-18B gels over a 24-h period. These test gels significantly decreased SLS absorption when compared to unprotected skin control samples (Table 2). The percent protection effect of three test gels against SLS percutaneous absorption was 88%, 81%, and 65%, respectively (Fig. 11).

CONCLUSIONS

1. Some BCs reduce the local damage due to various irritants and allergens.
2. Using inappropriate BC may enhance irritation rather than provide benefit.
3. In vitro methods are recommended in screening procedures for barrier cream candidates because of their simplicity, speed, and safety.

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Table 2 Cumulative Amount of [^{35}S]-SLS and Protection Effect Percent

	Control	A	B	C
Receptor fluid	0.43 ± 0.4	0.05 ± 0.05**	0.08 ± 0.05**	0.15 ± 0.2*
Skin content	14.19 ± 11.1	5.3 ± 7.3	3.95 ± 4.1	4.7 ± 3.5
Skin wash	78.6 ± 12.7	83.81 ± 12.0	83.19 ± 14.9	83.76 ± 10.6
% Protection effect		88%	81%	65%

Statistical differences in comparison with the control. *$p < 0.05$; **$p < 0.01$. Values are mean ± s.d. of percent of applied dose.
Source: Modified from Ref. 25.

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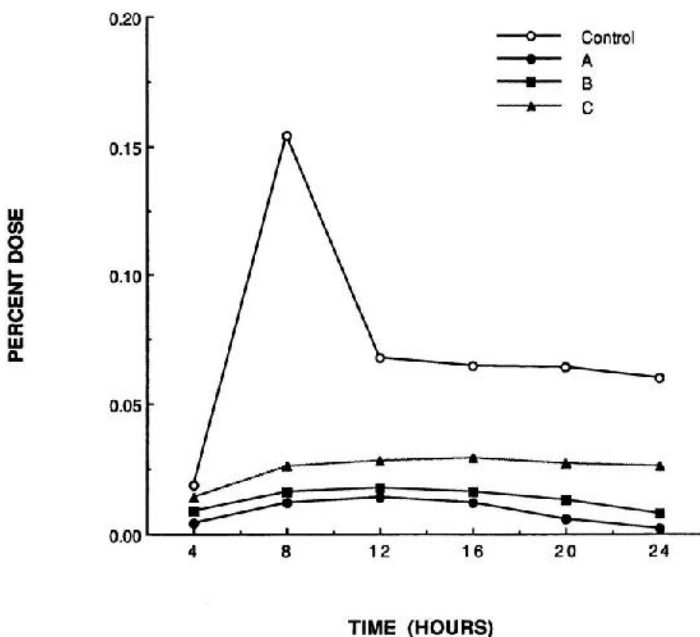

Figure 11 The cumulative amount absorbed into control skin and skin treated with three gels during 24-h exposure. Values are means of percent of applied dose. (Modified from Ref. 25.)

4. Animals may be used to generate kinetic data. Percutaneous absorption in pigs and monkeys shows a closer similarity to that in humans. But no animal, with its complex anatomy and biology, can simulate human absorption for all compounds. Therefore, the best estimate of human percutaneous absorption is determined by in vivo studies in humans.

5. Noninvasive bioengineering techniques are valuable in quantifying the inflammation response to various irritants and allergens when BCs are to be evaluated.

6. The accuracy of measurements of the efficacy of BCs depends on the use of proper methodology.

7. Above all, the clinical efficacy of BCs should be assessed in real rather than in experimental circumstance.

In the end, despite the power of these models, well-controlled field trials are required to define the relationship of the model to the occupational setting.

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Nevertheless, appropriate use of models should lead to formulation refinement and greater insight.

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Contact Urticaria Syndrome and Claims Support

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INTRODUCTION

Contact urticaria syndrome (CUS) was first defined by Maibach and Johnson (1) and, since then, numerous reports of contact urticaria to a variety of compounds such as foods, preservatives, fragrances, plant and animal products, metals and other things, continue to be reported. Therefore, it is important to determine, in a scientific manner, whether and in what dose a particular substance causes contact urticaria. Accurate experimental models are required to document urticaria-inducing properties of a substance; protocols to quantify efficacy of formulations that putatively inhibit CUS are also proposed. This chapter outlines current scientific knowledge and approaches to experimental methodology.

SYMPTOMS AND SIGNS

Immediate contact reactions, such as contact urticaria, appear within minutes to about 1 h after exposure of the urticariant to the skin. The patient may complain of local burning, tingling or itch, and swelling and redness may be seen (wheal and flare). Symptoms may extend extracutaneously, inducing, for example, bronchial asthma. In the most severe cases, anaphylactoid reactions may occur. A staging system of CUS has been described (see Table 1).

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Table 1 Staging of Contact Urticaria

	Cutaneous reactions only
Stage 1:	Localized urticaria (redness and swelling)
	Dermatitis (eczema)
	Nonspecific symptoms (itching, tingling, burning)
Stage 2:	Generalized urticaria
	Extracutaneous reactions
Stage 3:	Bronchial asthma (wheezing)
	Rhinitis, conjunctivitis (runny nose, watery eyes)
	Orolaryngeal symptoms (lip swelling, hoarseness, difficulty swallowing)
	Gastrointestinal symptoms (nausea, vomiting, diarrhea, cramps)
Stage 4:	Anaphylactiod reactions (shock)

Source: Ref. 11.

EPIDEMIOLOGY

Kanerva et al. (2,3) gathered statistical data on occupational contact urticaria in Finland. The incidence more than doubled from 89 reported cases in 1989 to 194 cases in 1994. From 1990 to 1994, a total of 815 cases was reported. The most common causes were, in decreasing order, cow dander, natural rubber latex (NRL), and flour/grains/feed. These three groups comprised 79% of all cases. Reflecting this, the most affected occupations (per 100,000 workers) were bakers, processed food preparers, and dental assistants, in decreasing order. Contact urticaria, therefore, is a common problem that may affect many people in the course of their daily lives.

MECHANISMS OF CONTACT URTICARIA

CUS can be described in two broad categories: nonimmunological contact urticaria (NICU) and immunological contact urticaria (ICU). The former does not require presensitization of the patient's immune system to an allergen, whereas the latter does. There are, however, contact urticaria reactions of unknown mechanism, and these are unclassified.

Nonimmunological Contact Urticaria

NICU is the most frequent immediate contact reaction (4) and occurs, without prior sensitization, in most exposed individuals. The symptoms may vary according to the site of exposure, the concentration, the vehicle, the mode of exposure, and the substance itself (5).

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The mechanism of NICU is not well understood. It was previously assumed that histamine was released from mast cells in response to exposure to an eliciting substance. However, the H_1 antihistamines—hydroxyzine and terfenadine—do not inhibit NICU to benzoic acid, cinnamic acid, cinnamic aldehyde, or methyl nicotinate in prick tests, but they do inhibit reactions to histamine itself (5,6). Therefore, mechanisms that do not involve histamine may mediate NICU for these substances.

Evidence suggests that prostaglandins may mediate NICU. Oral and topical nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit nonimmunological reactions (7). Lahti et al. (6), used laser Doppler flowmetry to demonstrate a reduction in NICU-induced erythema in subjects pretreated with NSAIDs. This group believed that inhibition of prostaglandin metabolism may explain this effect.

Supporting this, Morrow et al. (8) demonstrated an increase in plasma PGD_2 following the topical application of 1% sorbic acid to the human forearm. The time course of PGD_2 peaks correlated temporally with the observed intensity of cutaneous vasodilatation. Notably, histamine and PGE_2 levels at peak erythema were not significantly higher than pretreatment levels. This suggests that the release of vasodilatory prostaglandins induced by sorbic acid was selective for PGD_2, and that histamine is not involved in sorbic acid contact urticarial reactions. The release of PGD_2 was a dose-dependent effect, increasing with greater concentrations of sorbic acid, until reaching a plateau between 1 to 3%. Pretreating the subjects with oral aspirin (325 mg b.i.d. for 3 days) attenuated the observed cutaneous vasodilatation and inhibit release of PGD_2. In later studies, based on the same model, this group demonstrated similar results with benzoic acid–and nicotinic acid–induced contact urticaria (9,10).

These studies add evidence to the argument that prostaglandin metabolism is significant in the pathophysiology of CUS. Also, they not only suggest that NSAIDs are useful as a treatment but also that experimental subjects should avoid these drugs when participating in a contact urticaria study.

Ultraviolet A and ultraviolet B light also inhibits immediate nonimmunological contact reactions. Notably this effect can last for 2 weeks after irradiation and inhibits skin sites that were not directly irradiated (7). The authors suggest that there may be a systemic effect rather than simply a local one; however, the mechanism by which ultraviolet light inhibits NICU is not known.

Immunological Contact Urticaria

ICU is less frequent in clinical practice than the NICU form. It is a type 1 hypersensitivity reaction mediated by IgE antibodies, specific to the eliciting substance (11). Therefore, prior immune (IgE) sensitization is required for this type of contact urticaria.

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This sensitization can be at the cutaneous level, but also via mucous membranes, for example, in the respiratory or gastrointestinal tracts. Notably, ICU reactions may spread beyond the site of contact and progress to generalized urticaria and, in the most severe case, to anaphylactic shock.

People with an atopic background (personal or family background of eczema, hayfever, or asthma) are predisposed toward the immunological form of contact urticaria.

A well-studied example of ICU is allergy to natural rubber latex (NRL), which is found in a wide variety of products, such as balloons, condoms, and, importantly, surgical or protective gloves. ICU to NRL is a major occupational hazard in occupations that utilize such gloves (e.g., the health care profession).

Typically, latex gloves cause a wheal-and-flare reaction at the site of contact. This can affect either the person wearing the gloves or the person being touched by the wearer. In a study of 70 German patients with contact urticaria, 51% suffered rhinitis, 44% conjunctivitis, 31% dyspnea, 24% systemic symptoms, and 6% severe systemic reactions during surgery (12). In addition to direct skin contact, allergy may be caused by airborne NRL (13). Clearly, sensitized, yet undiagnosed, individuals are at risk when contacting ICU allergens.

Cross allergy can also induce ICU reactions: the patient may be sensitized to one protein but reacts to other proteins that contain the same (or similar) allergenic molecule. In the example of latex allergy, patients may also experience symptoms from banana, chestnut, and avocado (14). This phenomenon places ICU patients at further risk.

SITE SPECIFICITY OF CONTACT URTICARIA REACTIONS

Characteristics of the skin and also of its sensitivity to urticariants vary from site to site. This is an important consideration in experimental design, discussed below, and in diagnosis. Shriner and Maibach (15) used laser Doppler flow to map the regions of the human face most sensitive to NICU induced by benzoic acid: the neck was the most sensitive area, followed by the perioral and nasolabial folds. The least sensitive area was the volar forearm. The authors conclude that the neck, nasolabial, and perioral areas are the most sensitive to test for potential NICU to this agent. Lahti (5) found that the back was more sensitive than the hands, ventral forearms, or the soles of the feet, in his study of benzoic acid sensitivity at various body sites.

HUMAN EXPERIMENTAL PROTOCOLS

Human subjects may be used to determine the potential for a product to cause CUS in the human population. The protocols for ICU and NICU are the same,

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although ICU requires volunteers who are presensitized to the product. Subject selection, dosing, test site, application methods, and analysis are discussed in this section.

Subject Selection

To test a product for use in the general population, it is desirable to recruit a random pool of volunteers. However, this may introduce several confounding factors, such as age, skin disease, atopic tendency, and medication (such as NSAIDs) that may alter the results. Therefore, subjects must be chosen with particular regard to the aim of the study and screened carefully for inclusion and exclusion criteria, and for possible confounding factors.

Spriet et al. (16) suggest that subjects can be considered in three categories: serious sufferers, symptomatic volunteers, and healthy volunteers. It is likely that the latter are most suitable for testing new products, whereas the former two groups may be better suited to ICU studies or investigating claims that a product already in use causes CUS. Ideally, subjects should be representative of the population at which the product is aimed.

Site Selection

In the diagnostic investigation of a patient, the site affected in the patient's history may be tested. However, in a new product test trial, it is preferable to test the site at which the product is to be used. However, this may not be convenient for volunteers, and so concealed sites such as the volar aspect of the forearm or the upper back, may be chosen. Importantly, the site selected should be consistent in patients and controls, as different areas of the skin may demonstrate different sensitivities to the urticariant, thereby distorting comparability of the data. As noted above, different areas of the skin have varying capacity to induce urticaria, which should be considered when a site is chosen. Even in ICU, different skin sites may vary in their ability to elicit contact urticaria (17).

A history of skin disease may also affect the result. A test that is negative in nondiseased skin may in fact be positive in previously diseased or currently affected skin (18). If the initial studies are negative, it may be desirable to select subjects who are symptomatic and use the affected sites to test the substance.

Paired Comparison Studies

Paired comparison studies allow rapid comparison between treated and untreated groups. Randomized matched pairs can be grouped for treatment and control, or the subjects can be used as their own controls by applying the test substance and controls on separate sites. The latter is preferred, because each subject may have

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several doses applied to their skin, providing more data from a smaller pool of subjects. Furthermore, this decreases intersubject variation and confounding, thus providing better control.

Serial Doses

Performing studies at different doses of the product will allow the investigator to build a dose–response profile. This may indicate a minimum dose that causes a threshold response in the study group and also the dose at which a maximum response is seen. Extrapolating these data to the general population may give manufacturers an indication of a safe concentration for an ingredient to be included in a product. Dose–response analysis may also demonstrate that there is no safe concentration for that ingredient, or, indeed, that there is relatively little risk.

Examples of concentrations that have been used in dilution series in alcohol vehicles are 250, 125, 62, 31 mM for benzoic acid and 50, 10, 2, 0.5 mM for methyl nicotinate (7).

Application Techniques

Commonly used topical application techniques in both immunological and nonimmunological contact urticaria are the *open test* and the *chamber test.* A *use test* can be employed in known sufferers. A positive reaction comprises a wheal-and-flare reaction and sometimes an eruption of vesicles.

1. In the *open test*, 0.1 mL of the test substance is spread over a 3 × 3-cm area on the desired site. Lahti (7) suggests that using alcohol vehicles, with the addition of propylene glycol, enhances the sensitivity of this test compared with previously used petrolatum and water vehicles. The test is usually read at 20, 40, and 60 min, in order to see the maximal response. Immunological contact urticaria reactions appear within 15 to 20 min, and nonimmunological ones appear within 45 to 60 min after application (11).
2. The *chamber test* is an occlusive method of applying the substance to be tested. These are applied in small aluminum containers (Finn Chamber, Epitest Ltd., Hyrylä, Finland) and attached to the skin via porous tape. The chambers are applied for 15 min, and the results are read at 20, 40, and 60 min. The advantages of this method are that occlusion enhances percutaneous penetration, and therefore possibly the sensitivity of the test; also, a smaller area of skin is required than in an open test. For unexplained reasons, this occlusion may provide less responsivity than in the open test.

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3. The *use test* is a method in which a subject known to be affected uses the substance in the same way as when the symptoms appeared (e.g., putting surgical gloves on wet hands provokes latex ICU).

Other techniques, used in the assessment of ICU are the prick test, the scratch test, and the chamber prick test. RAST can be used to determine cross-reactivity (11,13).

CUS Inhibition

The above models can be employed to test the capability of a substance to inhibit CUS. This may be by topical application or by systemic means. Topical putative inhibitors can be studied by the paired comparison method, using multiple test sites and a control on the same subject. This allows serial dosing, with either the urticariant or the inhibitor, to identify its protective potential against a known urticariant. In systemic studies of an oral putative CUS inhibitor, for example, subjects can be randomized into matched pairs for treatment and control. Following systemic administration, a known urticariant can be applied topically in various doses, as outlined above, and the response assessed.

CLINICAL ASSESSMENT AND QUANTITATIVE METHODS

Previously, dermatological studies of the skin have scored the degree of urticaria by means of visual assessment by an experienced observer, usually a dermatologist. There are several advantages and disadvantages to this technique. Advantages are that it is inexpensive, visual scoring is rapid, subjects are regularly assessed so that the study can be curtailed if adverse reactions are severe, and unexpected findings can be handled by the investigator. However, simple observation may introduce error, inter- and intraobserver variation. This is especially important in larger studies, which may involve a team of investigators.

Visual observations are also often graded on an ordinal (nonlinear) scale (e.g., rating reactions as weak, moderate, or severe). As these data are not in linear numerical form, that statistical analysis is not as powerful as for quantitative data. In many studies, subjects report symptoms, also on an ordinal scale; this, again, is a subjective analysis prone to variation error.

In contrast, a quantitative analysis may provide linear numerical data that are easily reproducible and accurate in standardized conditions. Rather than providing a score, measured data allow for statistical comparison such as mean values and standard deviations. This adds to our understanding of the properties of the test substance. Thus, objective measurements can clearly benefit dermatology studies.

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Table 2 Scale to Score Erythema

Score	Description
1+	Slight erythema, either spotty or diffuse.
2+	Moderate uniform erythema.
3+	Intense redness.
4+	Fiery redness with edema.

Source: Ref. 18a.

Visual Scoring of Contact Urticaria

Contact urticaria can be graded visually by marking the degree of erythema and edema on an ordinal scale. Tables 2 and 3 provide examples.

Measurement of Erythema

Erythema, redness of the skin, is part of the skin inflammatory response that reflects localized increase in capillary blood flow elicited. Therefore, erythema can be measured by both the redness and the blood flow in the inflamed area.

Measuring Color

Two techniques have been used to measure color: remittance spectroscopy and tristimulus chromametry. Elsner gave detailed descriptions of the two techniques (19,20). Essentially, both methods detect light remitted from illuminated skin. Remittance spectroscopy employs multiple sensors to ‘‘scan’’ the light over the whole visible spectrum, producing a spectrogram. This differs from a tristimulus chromameter, in which the remitted light is transmitted to three photodiodes, each with a color filter with a specific spectral sensitivity: 450 nm (blue),

Table 3 Scale to Score Edema

Score	Description
1	Slight edema, barely visible or palpable.
2	Unmistakable wheal, easily palpable.
3	Solid, tense wheal.
4	Tense wheal, extending beyond test area.

Source: Ref. 18b.

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550 nm (green), 610 nm (red). The data from a colorimeter are expressed as a color value.

Remittance spectroscopy has been used to measure erythema in contact urticaria (21,22). This group evaluated remittance spectroscopy compared to visual scoring in the assessment of urticarial prick test reactions. They found that there was a significant difference between negative and positive reactions, and between positive and strong positive reactions (+/++). Baseline skin had an erythema index of 36, compared to 72 for a positive reaction. Negative skin sites had a slightly, but not significantly, raised erythema index, resulting from a dermographic reaction related to the procedure of the test itself. Notably, remittance spectroscopy was not as effective in discerning between the stronger reactions (++/+++), possibly because of the reduction of blood flow and hemoglobin content associated with the whitening of the center of the lesion and also because the blood flow may already have been maximized.

Laser–Doppler Blood Flowmetry

Several studies have identified a reliable correlation between skin blood flow measured by laser–Doppler flowmetry (LDF) and cutaneous inflammation (23–27). Bircher (28) reviews the use of LDF to study the role of various mediators in altering cutaneous blood flow.

The LDF technique measures the Doppler frequency shift in monochromatic laser light backscattered from moving red blood cells. This shift is proportional to the number of erythrocytes times their velocity in the cutaneous microcirculation. This noninvasive technique measures a surface area of 1 mm^2 and a depth of 1 to 1.5 mm. The 1-mm depth will therefore measure the upper horizontal plexus, consisting of arterioles, capillaries, and postcapillary venules. LDF does not measure the deep horizontal plexus that lies at the subcutaneous dermal junction. Detailed review of the principles, techniques, and methodology can be found in Berardesca et al. (22).

The changes in blood flow can be expressed in two ways. Either as the net change in cutaneous blood flow over the time of the experiment, which is given by the area under the curve (AUC), or as the maximal increase in flow over the baseline value (PEAK). Following a measurement of baseline blood flow, the product can be applied and posttreatment flow can be measured. The change in blood flow provides an indication of the degree of inflammation caused.

Measurement of Edema

Ultrasound has been used to quantify the edema component of urticaria. Agner and Serup (29) demonstrated a significant difference in skin thickness compared to controls in irritant reactions to sodium lauryl sulfate, nonanoic acid, and hydro-

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chloric acid. Serup et al. (30) used ultrasound to measure edema in patch tests, expressed in millimeters. Agner (31) suggests that A-mode ultrasound scanning is a simple, reproducible method of measuring skin thickness. One disadvantage, however, is that the technique is dependent on an experienced operator, which can potentially introduce observer error.

ANIMAL EXPERIMENTAL PROTOCOLS

Animal models are potentially useful to identify putative contact urticariants.

NICU

The guinea pig ear lobe resembles human skin in its reaction to contact urticariants (7,32), and is an established model for NICU. A positive reaction is seen as erythema and swelling of the ear, which can be quantified by measuring the thickness of the ear.

ICU

Laurema et al. (33) considered a possible animal model for ICU, topically presensitizing mice to trimellitic anhydride (TMA), which is known to cause IgE-mediated reactions. Topical TMA was applied to the dorsum of mice ears 6 days after they had been sensitized, eliciting a biphasic ear-swelling response. However, further studies are required to validate this model.

CONCLUSION

In conclusion, study of contact urticaria is possible with both human and animal subjects in whom a combination of subjective and objective analysis can identify potential immunological and nonimmunological contact urticariants.

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