# 13
# *Seborrheic Dermatitis (Dandruff)*

**Jan Faergemann**
*Sahlgrenska University Hospital, Gothenburg, Sweden*

Dandruff and seborrheic dermatitis are often mentioned together. Dandruff is the mildest manifestation of seborrheic dermatitis and it cannot be separated from seborrheic dermatitis. Therefore, what is mentioned in the literature for seborrheic dermatitis is also true for dandruff and vice versa. Seborrheic dermatitis is characterized by inflammation and desquamation in areas with a rich supply of sebaceous glands, namely, the scalp, face, and upper trunk (1). It is a common disease and the prevalence ranges from 2 to 5% in different studies. It is more common in males than in females. The disease usually starts during puberty and is more common around 40 years of age. Seborrheic dermatitis is characterized by red scaly lesions predominantly located on the scalp, face, and upper trunk. The skin lesions are distributed on the scalp, eyebrows, nasolabial folds, cheeks, ears, presternal and interscapular regions, axillae, and groin. Around 90 to 95% of all patients have scalp lesions and lesions on glabrous skin are found in approximately 60% of the patients. The lesions are red and covered with greasy scales. Itching is common in the scalp. Complications include lichenification, secondary bacterial infection, and otitis externa. The course of seborrheic dermatitis tends to be chronic with recurrent flare-up. A seasonal variation is observed with the majority of patients being better during the summertime. Mental stress and dry air are factors that may aggravate the disease. A genetic predisposition is an important factor. Seborrheic dermatitis is seen more frequently than expected in

patients with pityriasis versicolor, *Pityrosporum folliculitis*, Parkinson's disease, major truncal paralysis, mood depression, and acquired immunodeficiency syndrome (1).

## ETIOLOGY AND PATHOGENESIS

There are now many studies indicating that *Pityrosporum ovale* (*Malassezia*) plays an important role in seborrheic dermatitis (2). Many treatment studies describe the effectiveness of antimycotics, which reduces the number of *P. ovale*; recolonization leads to a recurrence of seborrheic dermatitis. The increased incidence of seborrheic dermatitis in patients with immunosuppressive disorders suggests that the relationship between *P. ovale* and the immune system is of importance.

*P. ovale* can activate complement by both the classic and alternative pathway (3). The humoral immune response to *P. ovale* in patients with seborrheic dermatitis and pityriasis versicolor has been studied using different antigen preparations and different techniques (3,4). Elevated titers in patients compared to controls as well as no difference in titers have been reported (3,4). In patients with seborrheic dermatitis, a reduced lymphocyte transformation response compared to healthy controls has been reported in two studies (5,6). However, in another study an enhanced lymphocyte stimulation response compared to healthy controls was found (7). In two recently published studies, no difference in lymphocyte stimulation response was found between patients with seborrheic dermatitis and healthy controls (8,9). In an immunological screening of patients with seborrheic dermatitis, we have found low ($<0.7$) responses in lymphocyte transformation tests to PHA and ConA in 13 of 30 patients (10). However, a recent study was not able to confirm that (8). Ashbee et al. found a normal PHA stimulation response in patients with seborrheic dermatitis compared to controls (7). In an earlier study we found a normal, but in the lower range ($<1$) CD4:CD8 ratio in 26 out of 30 patients with seborrheic dermatitis (10). Ashbee et al. found a normal CD4:CD8 ratio in patients compared to controls (7). Kieffer et al. found a low CD4:CD8 ratio in 13 of 19 patients with seborrheic dermatitis (11).

In a study by Neuber et al., IL-2 and IFN-$\gamma$ production by lymphocytes from patients with seborrheic dermatitis was markedly depressed and IL-10 synthesis was increased after stimulation with *P. ovale* extract (5). In another paper by Kesevan et al., the *Pityrosporum* yeast suppressed the production of the proinflammatory cytokines IL-1$\beta$, IL-6, and TNF-$\alpha$ (12).

In an immunohistochemical study in patients with seborrheic dermatitis deposits of complement C3c and IgG were found in the stratum corneum below

clusters of *P. ovale* (13). The local immune response in the skin may be different from the results obtained from in vitro studies on peripheral blood mononuclear cells and may better explain the inflammatory skin reaction seen in seborrheic dermatitis. In a recently fulfilled immunohistochemical study (data are still unpublished), we found an increase in all cellular markers in both lesional and nonlesional skin from patients with seborrheic dermatitis. We found an increase in markers for NK1 and CD16 positive cells (markers for natural killer cell function) as well as an increase in complement staining indicating that an irritant or nonimmunological stimulation of the immune system is important in seborrheic dermatitis. The reaction that we saw with the interleukins was complex, showing both an increase in the production of the inflammatory interleukins IL-1$\alpha$, IL-1$\beta$, IL-6, and TNF-$\alpha$, as well as interleukins responsible both for a Th1 and a Th2 reaction. It is important that no major differences were seen in the number of interleukin-associated cells between lesional and nonlesional skin in seborrheic dermatitis. However, the intercellular staining was more intense in lesional skin. The staining was also much higher in patients compared to healthy controls. The immune response in the skin of patients with seborrheic dermatitis is complex, but showed some similarities with the results obtained with *Candida* infections (14,15). *P. ovale* is a member of the normal skin flora and all individuals have both a humoral and a cellular immune response to this yeast (3,8). This is probably one of the important explanations why the immune response in the skin is more complex with diseases where this organism is involved. A strong stimulation of cells with natural killer function and complement activity may partly be explained by various enzymes (e.g., lipases) produced by *P. ovale* but further studies are needed to clarify this.

## TREATMENT

Seborrheic dermatitis is a chronic disease and patients should be informed about the risk for relapse and predisposing factors. Stress and winter climate have a negative effect on the majority of patients and summer and sunshine have a positive effect. In patients with neurological diseases and especially in patients with immunosuppressive disorders, seborrheic dermatitis is more resistant to therapy. In a young individual with resistant lesions always think of HIV infection. Mild corticosteroids are effective in the treatment of seborrheic dermatitis. However, the disease recurs quickly often within a few days. Antifungal therapy is effective in the treatment of seborrheic dermatitis and, because it reduces the number of *P. ovale*, the time to recurrence is increased compared to corticosteroids. Antifungal therapy should be the primary treatment for this disease.

Antifungal therapy for *P. ovale* is effective in treating most cases of seborrheic dermatitis and prophylactic treatment with antifungal drugs reduces the recurrence rate much more than corticosteroids (2,16–25). In one study, the combination of hydrocortisone and miconazole in an alcoholic solution was significantly more effective than hydrocortisone alone in reducing the number of *P. ovale* and the recurrence rate was also significantly lower with the combination therapy; 16% with the combination compared to 82% for hydrocortisone alone (2).

Ketoconazole is very effective in vitro against *P. ovale* with minimum inhibitory concentrations (MICs) in the range of 0.02 to 0.5 μg/mL. Oral ketoconazole has been effective in a double-blind, placebo-controlled trial in patients with seborrheic dermatitis of the scalp and other areas (18). However, oral ketoconazole should be reserved for patients not responding to topical therapy. In another double-blind, placebo-controlled study, ketoconazole 2% cream has been effective in the treatment of seborrheic dermatitis of the scalp and face (17), and in a comparative study between ketoconazole and hydrocortisone cream no difference was seen in effectiveness (20).

Ketoconazole shampoo used twice weekly is very effective in treating seborrheic dermatitis of the scalp (18). In a double-blind placebo-controlled study of ketoconazole shampoo used twice weekly for 4 weeks, 89% in the ketoconazole group was cured, compared with only 14% in the placebo group (18). Ketoconazole used once weekly has also been effective in preventing recurrence of dandruff in previously treated patients. Ketoconazole shampoo has been compared to ciclopirox olamine shampoo in the treatment of seborrheic dermatitis/dandruff (24). Both shampoos were equally effective and significantly more effective than placebo. However, at a follow-up visit 2 weeks after cessation of treatment, the recurrence rate was significantly lower in the ketoconazole group compared to the ciclopirox olamine group (24).

Other topical antimycotics are effective in the treatment of seborrheic dermatitis (2,16,21–25). Shampoos containing zinc pyrithione (21), selenium sulfide (16), or bifonazole (25) are also effective and widely used. Propylene glycol solution and shampoo has also been used successfully (22).

In severe inflammatory seborrheic dermatitis, topical treatment with antifungal therapy alone may not be effective. Some of these patients respond well to oral ketoconazole or itraconazole. Another therapy that can be effective is to combine potent topical corticosteroids with topical antifungal therapy. After clearance, many of these patients will remain free of lesions on prophylactic topical antifungal treatment. When lesions are covered with thick adherent scales, keratolytic therapy, especially in the scalp, is necessary. Seborrheic dermatitis especially in the scalp and external ear canal may be secondarily infected with bacteria. Often, in these patients, topical or oral antibacterial therapy in combination with regular treatment is indicated.

## REFERENCES


1. Burton JL, Holden CA. Seborrhoeic dermatitis. In: Champion RH, Burton JL, Burna DA, Breatnach SM, Rook A, Wilkinsson DS, Ebling FJG, eds. Textbook of Dermatology, (6th ed.). Oxford: Blackwell Scientific Publications, 1998:638–643.
2. Faergemann J. Seborrhoeic dermatitis and *Pityrosporum orbiculare*: Treatment of seborrhoeic dermatitis of the scalp with miconazole-hydrocortisone (Dactacort), miconazole and hydrocortisone. Br J Dermatol 1986; 114:695–700.
3. Bergbrant I-M. Seborrhoeic dermatitis and *Pityrosporum ovale*: cultural, immunological and clinical studies. Acta Derm Venereol (Stockh) 1991; (Suppl):167.
4. Midgley G, Hay RJ. Serological responses to *Pityrosporum* (*Malassezia*) in seborrhoeic dermatitis demonstrated by ELISA and Western blotting. Bull Soc Fr Mycol Méd 1988; 17:267–276.
5. Neuber K, Kröger S, Gruseck E, Abeck D, Ring J. Effects of *Pityrosporum ovale* on proliferation, immunoglobulin (IgA, G, M) synthesis and cytokine (IL-2, IL-10, $IFN_{\gamma}$) production of peripheral blood mononuclear cells from patients with seborrhoeic dermatitis. Arch Dermatol Res 1996; 288:532–536.
6. Wikler JR, Trago E, de Haan P, Nieboer C. Cell-mediated deficiency to *Pityrosporum orbiculare* in patients with seborrhoeic dermatitis. Abstracts of the ESDR-JSID-SID Tricontinental meeting, Washington DC, April 26–30, 1989.
7. Ashbee HR, Ingham E, Holland KT, Cunlife WJ. Cell-mediated immune response to *Malassezia furfur* serovars A, B and C in patients with pityriasis versicolor, seborrhoeic dermatitis and controls. Exp Dermatol 1994; 3:106–112.
8. Bergbrant I-M, Andersson B, Faergemann J. Cell-mediated immunity to *Pityrosporum ovale* in patients with seborrhoeic dermatitis and pityriasis versicolor. Clin Exp Dermatol 1999; 24:402–406.
9. Pearry ME, Sharpe GR. Seborrhoeic dermatitis is not caused by an altered immune response to *Malassezia* yeast. Br J Dermatol 1998; 139:254–263.
10. Bergbrant I-M, Johansson S, Robbins D, Scheynius A, Faergemann J, Söderström T. An immunological study in patients with seborrhoeic dermatitis. Clin Exp Dermatol 1991; 16:331–338.
11. Kieffer M, Bergbrant I-M, Faergemann J, Jemec GB, Ottevanger V. Skov. Immune reactions to *Pityrosporum ovale* in adult patients with atopic dermatitis and seborrhoeic dermatitis. J Am Acad Dermatol 1990; 22:739–742.
12. Kesavan S, Walters CE, Holland KT, Ingham E. The effects of *Malassezia* on proinflammatory cytokine production by human peripheral blood mononuclear cells in vitro. Med Mycol 1998; 36:97–106.
13. Piérard-Franshimont C, Arrese JE, Piérard GF. Immunohistochemical aspects of the link between *Malassezia ovalis* and seborrhoeic dermatitis. J Eur Acad Dermatol Venereol 1995; 4:14–19.
14. Romani L, Bistoni F, Puccetti P. Initiation of T-helper cell immunity to *Candida albicans* by IL-12: the role of neutrophils. Chem Immunol 1997; 68:110–135.
15. Gulay Z, Imir T. Anti-candidial activity of natural killer (NK) and lymphokine activated killer (LAK) lymphocytes in vitro. Immunobiology 1996; 95:220–230.

16. Shuster S. The aetiology of dandruff and the mode of action of therapeutic agents. Br J Dermatol 1984; 111:235–242.
17. Skinner RB, Noah PW, Taylor RM, et al. Double-blind treatment of seborrheic dermatitis with 2% ketoconazole cream. J Am Acad Dermatol 1985; 2:852–857.
18. Faergemann J. Treatment of seborrhoeic dermatitis of the scalp with ketoconazole shampoo. Acta Dermatol Venereol (Stockh) 1990; 70:171–172.
19. Ford GP, Farr PM, Ive FA, et al. The response of seborrhoeic dermatitis to ketoconazole. Br J Dermatol 1984; 111:603–607.
20. Stratigos ID, Katamboas A, Antoniu CH, et al. Ketoconazole 2% cream versus 1% hydrocortisone cream in the treatment of seborrhoeic dermatitis: A double-blind comparative study. J Am Acad Dermatol 1988; 19:850–853.
21. Marks R, Pears AD, Walker AP. The effects of a shampoo containing zinc pyrithione on the control of dandruff. Br J Dermatol 1985; 112:415–422.
22. Faergemann J. Propylene glycol in the treatment of seborrheic dermatitis of the scalp: A double-blind study. Cutis 1988; 42:69–71.
23. Faergemann J. Treatment of seborrhoeic dermatitis with bifonazole. Mycoses 1989; 32:309–311.
24. Shuttleworth D, Squire RA, Boorman GC, Goode K. Comparative clinical efficacy of shampoos containing ciclopirox olamine (1.5%) or ketoconazole (2%; Nizoral) for the control of dandruff/seborrhoeic dermatitis. J Dermatol Treat 1998; 9:157–162.
25. Zeharia A, Mimouni M, Fogel D. Treatment with bifonazole shampoo for scalp seborrhea in infants and young children. Ped Dermatol 1996; 13:151–153.

# 14
# *Dermatotoxicology Overview*

**Philip G. Hewitt**
*Merck kGaA, Darmstadt, Germany*

**Howard I. Maibach**
*University of California, San Francisco, California*

## INTRODUCTION

Cosmeceuticals are presumably relatively ''safe.'' Adverse skin responses associated with repetitive, low-dose exposure to consumer products are all too often not accurately predicted by the required assays. The need to market products with low risk of producing dermal and systemic injury to increase consumer satisfaction has led to the development of numerous assays to rank chemicals for their ability to injure the skin. Although these assays are not routinely mandated by regulatory agencies for cosmetics and skin care, the frequency with which they are conducted and their utility warrant attention.

The field of dermatotoxicology includes measurement of absorption of materials as well as assays that evaluate the ability of topically applied chemicals to induce or promote the development of neoplasia, trigger an immune response in the skin, directly destroy the skin (corrosion), irritate the skin, produce urticaria (hives), and produce noninflammatory painful sensations. The inflammatory responses of skin are the most common chemically induced dermatoses in humans.

## DERMATOPHARMACOKINETICS: RELATION TO PREDICTIVE ASSAYS

Although the skin's barrier properties are impressive, it has been shown to be a major route of entry under some exposure situations. Interest in dermatopharmacokinetics has increased as the skin has been reconsidered to be a route for systemic administration of drugs and chemicals, as well as a route of entry for toxins. A variety of assays, both in vivo and in vitro, for measuring absorption through the skin, have been developed (1,2) and many factors that govern absorption through the skin have been determined.

A major diffusion barrier of the skin is considered to be the stratum corneum. Absorption of chemicals through shunts, openings of skin appendages, and gaps in the stratum corneum associated with these structures have been considered (3). Absorption can be described as passive diffusion across this membrane by the equation, $J = (K_m C_v D_m) \div \delta$ [rate of absorption = (vehicle/stratum corneum partition coefficient $\times$ skin surface concentration $\times$ diffusion constant of penetrant in stratum corneum) divided by thickness of stratum corneum (4)]. Other factors that affect thermodynamic activity of the solution at the skin surface (e.g., pH and temperature) may vary flux (5,6). Vehicle influence cannot be overstated; for a specific concentration of chemical, thermodynamic activity may vary by 1000-fold from one vehicle to another (6). Other factors that affect percutaneous absorption include condition of the skin (7), age, surface area to which the material is applied (8), penetrant volatility, temperature and humidity (9), substantivity, and wash-and-rub resistance to removal from the skin and binding to the skin (10).

Once a chemical has gained access to the viable epidermis, it may initiate a local effect, be absorbed into the circulation and produce an effect, or produce no local or systemic effects. The viable epidermis contains enzymes capable of metabolizing exogenous chemicals (11), including a substantial cytochrome P450 system, esterases, mixed-function oxidases, and glucuronyltransferases. Early studies conducted in vitro using whole skin indicated that enzymatic activity in skin was only a fraction of the activity of the liver. However, when the surface area of the epidermis is taken into account, then enzymatic activities of the epidermis can range from 80% to 240% of those in liver (12).

## IN VIVO PERCUTANEOUS ABSORPTION ASSAYS

Percutaneous absorption can be determined by applying a known amount of chemical to a specified surface area and then measuring levels of the chemical in the urine and/or feces. Because the analytical techniques to measure the chemical are not always available and because some chemicals may be metabolized, radiolabeled chemicals, $^{14}C$ or $^{3}H$, are often used.

In vivo studies have been conducted in humans and other species (12). Comparison of absorption rates of a number of compounds showed that absorption rates in the rat and rabbit tend to be higher than humans and that the skin permeability of monkeys and swine more closely resembles that of humans. No significant mouse–human skin comparisons exist. Guinea pig–human comparisons offer some promise for refinement of guinea pig–human irritation and sensitization extrapolations (13). Although these differences are not predicted by any single factor, they are not unexpected in light of differences in metabolism and in routes of excretion. Therefore, the metabolic capabilities of the species should be considered when selecting an animal model and designing the experiment. Although there is no question that pharmacokinetic studies of this type in humans or animals provide the best estimate of percutaneous absorption, the cost and difficulty in conducting well-controlled studies have led to the use of other in vivo assays that are poorer predictive tools and to the development of in vitro models.

## IN VITRO PERCUTANEOUS PENETRATION ASSAYS

The excised skin of humans or animals can be used to measure penetration of chemicals. In vitro assays using excised skin utilize specially designed diffusion cells (1,14,15). The skin is stretched over the opening of a collecting receptacle, epidermal side up. The chemical is applied to the epidermis and fluid from the receptacle is assayed to measure the penetration of the chemical. This type of in vitro assay offers some advantages over in vivo assays: highly toxic compounds can be studied in human skin, large numbers of cells can be run simultaneously, diffusion through the membrane (eliminating other pharmacokinetic factors) can be studied, and these assays may be easier to conduct.

Comparison of penetration rates obtained from in vitro and in vivo assays have been made (1), often with a good correlation; however, with some, correlation was poor. Differences in the methods for some compounds could be explained on the basis of solubilities in the receptacle fluid and blood; others could not be explained. Skin of the weanling pig and miniature swine appear to be good in vitro models for most compounds (2). Although a limited number of studies have been reported, the skin of monkeys also appears to be a good model (8). Rat skin appears to be a good model for some compounds; however, when differences have been noted, they have been large.

## ALLERGIC CONTACT DERMATITIS

Jadassohn (16) demonstrated that in some patients dermatitis was due to increased sensitivity following repeated contact with a substance and not the irritant proper-

ties of the material. By 1930, a procedure for producing this hypersensitivity to chemicals in guinea pigs had been developed (17). Landsteiner and associates demonstrated that low-molecular-weight chemicals conjugate with proteins to form an antigen that stimulates the immune system to form a hyperreactive state (18); immunogenicity is related to chemical structure (19); and two types of immunological response exist, one transferable by serum and another transferred by suspensions of white blood cells (20). These mechanisms are succinctly provided by von Blomberg (21).

Appropriate planning and execution of predictive sensitization assays is critical. The first priority is to choose an appropriate experimental design. A common error in choosing an animal assay is using Freund's complete adjuvant (FCA) when setting dose–response relationships. The adjuvant provides such sensitivity that dose–effect relationships are muted. Choice of dose and vehicle appropriate to the assay and the study question is the second priority. Although dose must be high enough to ensure penetration, it must be below the threshold at challenge to avoid misinterpretation of irritant inflammation as allergic. Knowing the irritation potential of compounds will allow the investigator to design and execute these studies appropriately. Vehicle choice determines in part the absorption of the test material and can influence sensitization rate, ability to elicit response at challenge, and the irritation threshold.

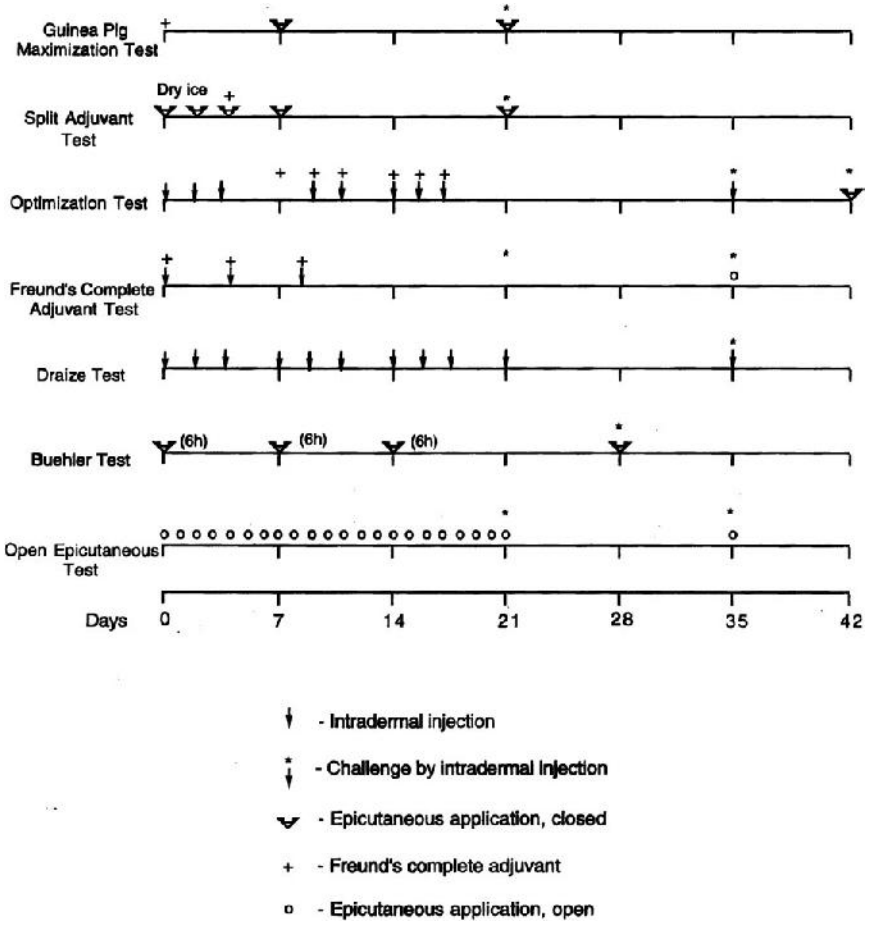
## QUANTITATIVE STRUCTURE ACTIVITY RELATIONSHIPS

Quantitative structure activity relationships (QSAR) describe a relationship of chemical structure to biological activity—in this case allergic contact dermatitis. A computer-assisted database describing the chemical structure and physicochemical parameters of an array of chemicals provides a facile approach to designing appropriate in vitro, animal, and human sensitization studies (22). In essence, searching the prior experimental data permits not only determination of relationship between structures and allergenicity, but provides insight into planning a given experiment. For example, if a closely related structure to the chemical of interest has been shown to be a potent allergen, the new chemical may be examined with a more quantitative assay.

## GUINEA PIG SENSITIZATION TESTS

Predictive animal tests to determine the potential of substances to induce delayed hypersensitivity in humans are conducted most often in guinea pigs. Several tests

**Table 1** Features of Most Commonly Used Assays to Predict Sensitization




have been described. All utilize young (1–3 months), randomly bred, albino guinea pigs. Most visually evaluate the responses using descriptive scales for erythema and edema. The tests differ significantly in route of exposure, use of adjuvants, induction interval, and number of exposures. The principal features of the most commonly used assays and assays acceptable to regulatory agencies to predict sensitization are summarized in Table 1 (23–25).

## DRAIZE TEST

The Draize sensitization test (DT) (26,27) was the first predictive sensitization test accepted by regulatory agencies. One flank of 20 guinea pigs is shaved and 0.05 ml of a 0.1% solution of test material in saline, paraffin oil, or polyethylene glycol is injected into the anterior flank on day 0. Every other day through day 20, 0.1 mL of the test solution is injected into a new site on the same flank. After a 2-week rest period, the opposite untreated flank is shaved and 0.05 mL of test solution is injected into each animal (challenge). Twenty previously untreated controls are injected at the same time. The test site is visually evaluated 24 h and 48 h after injection. A larger or more intensely erythematous response than that of controls is considered a positive response.

## OPEN EPICUTANEOUS TEST

The open epicutaneous test (OET) (28) simulates the conditions of human use by utilizing topical application of the test material. The procedure determines the doses required to induce sensitization and to elicit a response in sensitized animals. The irritancy profile is determined by applying 0.025 mL of varying concentrations to a 2 $cm^2$ area of the shaved flanks of six to eight guinea pigs. Test sites are visually evaluated 24 h after application of test solutions to erythema. The dose not causing a reaction in any animal (maximal nonirritant concentration) and the dose causing a reaction in 25% of the animals (minimal irritant concentration) are determined. During induction, test solution is applied to flank skin of six to eight guinea pigs for 3 weeks, or 5 times a week for 4 weeks. A control group is treated with vehicle only. The highest dose tested is usually the minimal irritant concentration and lower doses are based on usage concentration or a stepwise reduction. Twenty-four to 72 h after the last induction treatment, each animal is challenged on the untreated flank. The minimal irritant concentration, the maximum nonirritant concentration and five solutions of lower concentrations are applied. Skin reactions are read on an all-or-none basis at 24, 48, and 72 h after application. The maximum nonirritating concentration in the vehicle-treated group is calculated. Animals in test groups that develop inflammatory responses to lower concentrations are considered sensitized.

## BUEHLER TEST

The Buehler test (occlusion only) (29) also employs topical application. An absorbent patch, or vehicle alone, is placed on the shaved flanks of 10 to 20 guinea pigs. Test concentration varies from undiluted to usage levels. A concentration

that produces slight erythema is optimum and is selected based on an irritancy screen conducted in other animals. This procedure is repeated 7 and 14 days after the initial exposure. Two weeks after the last induction patch, animals are challenged with patches saturated with a nonirritating concentration of test material and with the vehicle. After 6 h, the patch is removed and the area depilated. Test sites are visually evaluated 24 and 48 h after patch removal. Animals developing erythematous responses are considered sensitized (if irritant control animals do not respond).

## FREUND'S COMPLETE ADJUVANT TEST

Freund's complete adjuvant test (FCAT) is an intradermal technique incorporating test material in a 50/50 mixture of FCA and distilled water. The description is summarized by Klecak (30).

## OPTIMIZATION TEST

The optimization test resembles the DT, but incorporates the use of adjuvant for some induction injections and both intradermal and topical challenges (27). On day 1, one injection into the shaved flank and one into a shaved area of dorsal skin are given. Two and 4 days later, one injection into a new dorsal site is given. The test material is administered in saline during the first week. During the second and third weeks, test material is administered in FCA/saline every other day to a shaved area over the shoulders. Twenty test animals are treated and 20 controls are injected with vehicle alone. Thickness of a skinfold over the injection site is measured with a caliper. Any animal developing a reaction volume at challenge greater than the mean plus 1 standard deviation during induction is considered sensitized. A second challenge is conducted 45 days after the first injection. A nonirritating concentration of the test material in a suitable vehicle is applied to the flank skin, away from injection sites. Reactions are visually evaluated after 24 h using the 4-point erythema scale of the Draize primary irritancy scale. To classify materials as strong/moderate/weak/nonsensitizer, a classification scheme has been devised using results of exact Fisher test and number of positives detected.

## SPLIT ADJUVANT TEST

The split adjuvant test (30) utilizes skin damage and FCA as adjuvants. An area of back skin of 10 to 20 guinea pigs is shaved to glistening, then treated with

dry ice for 5 to 10 s. A layer of loose mesh gauze and stretch adhesive with a $2 \times 2$ cm$^2$ opening over the shaved area is placed around the animal. Approximately 0.2 mL of creams or solid test material, 0.1 mL if liquid, is spread over the test site and occluded. The concentration tested varies by irritancy potential, use conditions, etc. Two days later, the occlusive filter paper is removed, the test material reapplied, and the covering replaced. On day 4, the filter paper cover is removed, two injections of 0.075 mL FCA are given into the edges of the test site, the test material reapplied, and the site resealed. On day 7, the test material is reapplied and on day 9 the dressing is removed. Twenty-two days after the initial treatment, animals are challenged by topical application of 0.5 mL of test material to a $2 \times 2$ cm$^2$ area of the shaved midback. A group of naive controls, 10 to 20 animals, is treated by the same procedure at challenge. Twenty-four, 48, and 72 h after application, the dressing is removed and the test site is visually evaluated using a descriptive visual scale. Sensitization of individual animals is indicated by significantly stronger reactions than those of controls.

## GUINEA PIG MAXIMIZATION TEST

The guinea pig maximization test (GPMT) (27,30) combines FCA, irritancy, intradermal injection, and occlusive topical application during the induction period. Two identical sets of 0.1-mL intradermal injections of 50/50 FCA/water, test material in water, paraffin oil, or propylene glycol and the same dose of test material in FCA/vehicle are placed on a filter paper, placed over the shaved injection site, covered with approximately $4 \times 8$ cm occlusive surgical tape, and secured in place. If the test material is nonirritating, the test site is pretreated with 10% sodium lauryl sulfate (SLS) in petrolatum on day 6 to provoke an irritant reaction. After 48 h, test and control (vehicle alone) animals are challenged on the shaved flank with the highest nonirritating concentration and with the vehicle. Solutions are applied to filter paper secured in place and patches removed 24 h later. Reactions are visually evaluated 24 and 48 h after patch removal. Reactions are considered positive when they are more intense than the response to vehicle and the responses to the test materials in controls. The test material is rated as a weak-to-extreme sensitizer, based on the incidence of positives in the test group (Table 2).

## HUMAN SENSITIZATION ASSAYS

Chemicals can be tested for their ability to induce contact hypersensitivity in panels of human volunteers from whom informed consent is obtained. Allergic contact dermatitis to materials already in commercial use is sometimes detected

**Table 2** Guinea Pig Maximization Test (GPMT) Rating of Weak-to-Extreme Sensitizers

| Sensitization rate (%) | Grade | Class |
|---|---|---|
| 0–8 | I | Weak |
| 9–28 | II | Mild |
| 29–64 | III | Moderate |
| 65–80 | IV | Strong |
| 81–100 | V | Extreme |

by early induction patches. This does not reflect the particular test material's ability to induce sensitization. It merely indicates that under patch conditions, the material may elicit a response in presensitized individuals.

There are four basic predictive human sensitization tests in current use: (1) a single induction/single challenge patch test; (2) repeated insult patch test (RIPT); (3) RIPT with continuous exposure (modified Draize); and (4) the maximization test, all of which use similar customized patches (31,32). Principal features of human sensitization assays are summarized in Table 3. For assays other than maximization, 150 to 200 subjects are usually tested. Henderson and Riley (33) statistically showed that if no positive reactions are observed in 200 randomly selected subjects, as many as 15/1000 of the general population may react (95% confidence). As sample size is reduced, the likelihood of unpredicted adverse reactions in the general population increases.

## REPEAT INSULT PATCH TESTS

In the Draize human sensitization test (34), an occlusive patch containing the test material is applied to the upper arm or upper back of 200 volunteers for 48 h. The test site is evaluated at patch removal for erythema and edema. This process is repeated until a total of 9 to 10 patches have been applied. Ten to 14 days after application of the last induction patches, subjects are challenged via a patch applied to a new site for 48 h. Sites are visually evaluated at removal of the patch and the response at challenge is compared to the response to patches applied early in induction.

## MODIFIED DRAIZE HUMAN SENSITIZATION TEST

The RIPT procedure was modified to provide continuous patch exposure to the test material during a 3-week induction period (35,36). Patches are applied to

**Table 3** Principal Features of Human Sensitization Assays

| Test | No. subjects | Concentration/ amount of test material | Vehicle | Skin site | Patch type | *Induction* No. patches | Duration | Rest | Challenge |
|---|---|---|---|---|---|---|---|---|---|
| Schwartz | 200 | Fabric | | | Fabric | 1 | 5 days | 10 days | 48-h patch; observe 10 days |
| Schwartz | 200 | 1-in. fabric, liquid or powder | | Arm, thigh or back | Cellophane covered with 2 × 2 in. Elastoplast | 1 | 72 h | 7–10 days | 72 h; same site; observe 3 days |
| ''Prophetic'' Schwartz-Peck | 200 | 1/4-in.$^2$ 4-ply gauze, liquid saturated[a] | Petrolatum or corn oil | Arm or back | 1-in.$^2$ nonwaterproof cellophane covered with 2-in.$^2$ adhesive plaster | 1 | 24, 72, or 96 h | 10–14 days | 48-h; observe 3 days: compare new and old formulas |
| ''Repeated insult'' Shelanski | 200 | Proportional to area of ultimate use | Mineral oil | | Occlusion: follows Schwartz test | 10–15 | 24 h every other day: same site | 2–3 weeks | 48-h patch |
| ''Repeated insult'' Draize | 100 males 100 females | 0.5 mL or 0.5 g | | Arm or back | 1 in.$^2$ | 10 | 24 h alternate days | 10–14 days | Repeat patch on new site |
| Modified Draize | 200 | 0.5 mL or 0.5 g high concentration | Petrolatum | Arm | Square BandAid, no perforations | 10 | 48 h | 2 weeks | Patch on new site 72 h with nonirritant concentration |
| ''Maximization'' (Kligman) | 25 | 1 mL 5% SLS[b], followed by 1 mL 25% test material | Petrolatum | Forearm or calf | 1.5-in.$^2$ Webril occluded with Blenderm. held with perforated plastic tape | 5 (same site) | 24 h SLS followed by 48-h test material for each of 5 inducing applications | 10 days | 1-in.$^2$ patch on lower back or forearm: 0.4 mL of 10% SLS for 1 h followed by 0.4 mL of 10% test material for 48 h |
| Modified ''maximization'' | 25 | Same as maximization | Petrolatum | Forearm or calf | Same as maximization | 7 | 24 h SLS followed by 48-h test material for each of 7 inducing applications | 10 days | 2% SLS for 0.5 h followed by 48-h patch with test material |

[a] Modified for solids, powders, ointment and cosmetics. Concentration, amount, area and site of application are considered important in evaluating results. Authors recommended that cosmetics be tested uncovered.

[b] Sodium lauryl sulfate (SLS) pretreatment is used to produce moderate inflammation. SLS is mixed with test material when compatible. SLS is eliminated when the test material is a strong irritant. Table modified from Patrick E, Maibach HI. Predictive skin irritation tests in animals and humans. In: Marzulli FN, Maibach HI, eds. Dermatotoxicology, 3rd ed. New York: Hemisphere Publishing 1991:201–222.

the outer upper arm each Monday, Wednesday, and Friday, until a total of 9 to 10 patches have been applied. Fresh patches are applied to the same site unless moderate inflammation has developed when the patches should be placed on adjacent noninflamed skin. This produces a continuous exposure of 504 to 552 h compared to a total exposure period of 216 to 240 h for RIPT of comparable induction periods. In addition, induction concentrations are increased to levels above usage exposure. Two weeks after induction, subjects are challenged by exposure of a new site to a patch for 48 to 72 h at a nonirritating concentration. Test sites are evaluated at 0 and 24 h after removal.

## IRRITANT DERMATITIS

Historically, skin irritation has been described by exclusion as localized inflammation not mediated by either sensitized lymphocytes or by antibodies (i.e., nonimmunogenic). Application of some chemicals directly destroys tissue, producing skin necrosis at the site of application (i.e., corrosive chemicals). Chemicals may disrupt cell functions and/or trigger the release, formation, or activation of autocoids that produce local increases in blood flow, increase vascular permeability, attract white blood cells in the area, or directly damage cells. The additive effects of these mediators result in local skin inflammation (i.e., acute irritants). A number of as-yet poorly defined pathways involving different processes of mediator generation appear to exist. Although no agent has yet met all the criteria to establish it as a mediator of skin irritation, histamine, 5–hydroxytryptamine, prostaglandins, leukotrienes, kinins, complement, reactive oxygen species and products of white blood cells have been strongly implicated as mediators of some irritant reactions (37).

Some chemicals do not produce acute irritation from a single exposure but may produce inflammation following repeated application to the same area of skin [cumulative irritation (38)]. Studies on skin corrosion are conducted in animals, using standardized protocols as it is not appropriate to conduct screening studies in humans. But acute irritation is sometimes evaluated in humans after animal studies have been completed. Tests for cumulative irritation in both animals and humans have been reported.

## IN VITRO ASSAYS

Numerous in vitro assays for irritation exist. Rougier et al. summarize these assays and offer guidelines as to their potential validation (39).

## IRRITATION TESTS IN ANIMALS

### Draize-Type Tests

Primary irritation and corrosion are most often evaluated by modifications of the method described by Draize (24). The Federal Hazardous Substance Act (FHSA) adopted one modification as a standard procedure (22). The backs of six albino rabbits are clipped free of hair. Each undiluted material is tested on two 1-in.$^2$ sites on the same animal (one site is intact and one is abraded in such a way that the stratum corneum is opened but no bleeding produced). Each test site is covered with two layers of 1-in.$^2$ surgical gauze and secured in place. The entire trunk of the animal is then wrapped with rubberized cloth or other occlusive impervious material to retard evaporation of the substances and hold the patches in position. Twenty-four and 48 h after application the wrappings are removed and the test sites evaluated for erythema and edema, using a prescribed scale. Modifications of the Draize procedure that have been proposed include changing the species tested (40), reduction of exposure period, use of fewer animals and testing on intact skin only (41). Several governmental bodies utilized their own modification of the Draize procedure for regulatory decisions. The FHSA, DOT, Environmental Protection Agency (EPA), Federal Insecticide, Fungicide, Rodenticide Act (FIFRA), and OECD guidelines are contrasted to the original Draize methods. All Draize-type tests are used to evaluate corrosion as well as irritation. When severe reactions that may not be reversible are noted, test sites are observed for a longer period. Delayed evaluations are usually made on days 7 and 14, but maybe as late as 35 days.

### Non-Draize Animal Studies

Animal assays to evaluate the ability of chemicals to produce cumulative irritation have been developed (42). Those assays used often are not as well standardized as Draize-type tests and many variables have been introduced by multiple investigators.

Repeat application patch tests in which diluted materials are applied to the same site each day for 15 to 21 days have been reported using several species (the guinea pig or rabbit being most commonly used) (42). Because the degree of occlusion is an important determinant of percutaneous penetration, the choice of covering materials may determine the sensitivity of a given test (43). A reference material of similar use or one that produces a known effect in humans is included in almost all repeat application procedures. Test sites are evaluated for erythema and edema, either using the scales of the Draize-type tests or more descriptive scales developed by the investigator.

## Human Irritation Tests

Because only a small area of skin need be tested, it is possible to conduct predictive irritation assays in humans, provided systemic toxicity (from absorption) is low. Human tests are preferred to animal tests in some cases because of the uncertainties of interspecies extrapolation. Many forms of a single application patch test have been published. Custom-made apparatus to hold the test material have been designed (29,43). Duration of patch exposure has varied between 1 and 72 h. The single application patch procedure outlined by the National Academy of Sciences (NAS) Publication 1138 (44) incorporates important aspects of assays. For new materials or volatiles, a relatively nonocclusive tape (e.g., Micropore, Dermical, or Scanpore) should be used. Increasing the degree of occlusion with occlusive tapes (e.g., Blenderm) or chamber devices generally increases the severity of responses. A 4-h exposure period was suggested by the NAS panel. However, it is desirable to test new materials and volatiles for shorter periods (30 min to 1 h) and many investigators apply materials intended for skin contact between 24- and 48-h periods. After the period of exposure, the patches should be removed and the area cleaned with water to remove any residue. Responses are evaluated 30 min to 1 h and 24 h (to allow hydration and pressure effects to subside) after patch removal. Persistent reactions may be evaluated for 3 to 4 days. The Draize scales for erythema and edema have no provision for scoring papular, vesicular, or bullous responses. Therefore, integrated scales ranging from 4 to 16 points have been published and are generally preferred to the Draize scales.

Most multiple application patch tests were patterned after human sensitization studies with 24-h exposures, with or without a rest period between patches. The early work of Kligman and Wooding (45) forms the basis for the irritant dose 50 ($ID_{50}$) comparative system.

The cumulative irritation assay (46) was used to compare antiperspirants, deodorants, and bath oils to provide guidance for product development. A 1-in.$^2$ patch of Webril was saturated with test compound and applied to the skin of the upper back. After 24 h, the patch was removed, the area evaluated, and a fresh patch applied. The procedure was repeated daily for up to 21 days. The $IT_{50}$ [as described by Kligman and Wooding (45)] was used to evaluate and compare test materials. Modifications of the cumulative irritation assay have been reported (44,47) and newer chamber devices have replaced Webril with occlusive tape by some. Many variables of the chosen test procedure (e.g., vehicle, type of patch, concentration tested) may modify the intensity of the response (48,49). Differences in intensity of responses have also been linked to differences in age (50), sex (50), and race (51).

## CONTACT URTICARIA SYNDROME

Contact urticaria syndrome (CUS) has been defined as a wheal-and-flare response that develops within 30 to 60 min after exposure of the skin to certain agents (52,53). Symptoms of immediate contact reactions can be classified according to their morphology and severity:

Itching, tingling, and burning with erythema is the weakest type of immediate contact reaction.
Local wheal and flare with tingling and itching represents the prototype reaction of contact urticaria.
Generalized urticaria after local contact is rare, but can occur from strong urticaria.
Symptoms in other organs can appear with the skin symptoms in cases of immunological contact urticaria syndrome.

The strength of the reactions may vary greatly and often the whole range of local symptoms can be seen from the same substance if different concentrations are used (54). In addition, a certain concentration of contact urticant may produce strong edema and erythema reactions on the skin of the upper back and face but only erythema on the volar surfaces of the lower arms or legs. In some cases, contact urticaria can be demonstrated only on damaged or previously eczematous skin and it can be part of the mechanism responsible for maintenance of chronic eczemas (25). Because of the risk of systemic reactions (e.g., anaphylaxis), human diagnostic tests should only be performed by experienced personnel with facilities for resuscitation on hand. Contact urticaria has been divided into two main types on the basis of proposed pathophysiological mechanisms, namely, nonimmunological and immunological (55).

## NONIMMUNOLOGICAL CONTACT URTICARIA

Nonimmunological contact urticaria (NICU) is the most common form and occurs without previous exposure in most individuals. The reaction remains localized and does not cause systemic symptoms to spread to become generalized urticaria. Typically, the strength of this type of contact urticaria reaction varies from erythema to a generalized urticarial response, depending on the concentration, skin site, and substance. The mechanism of nonimmunological contact urticaria has not been delineated, but a direct influence on dermal vessel walls or a nonantibody-mediated release of histamine, prostaglandins, leukotrienes, substance P, other inflammatory mediators, or different combinations of these mediators represents possible mechanisms (56). The most potent and best studied substances producing nonimmunological contact urticaria are benzoic acid, cinnamic

acid, cinnamic aldehyde, and nicotinic esters. Under optimal conditions, more than half of a random sample of individuals show local edema and erythema reactions within 45 min of application of these substances if the concentration is high enough.

## IMMUNOLOGICAL CONTACT URTICARIA

Immunological contact urticaria (ICU) is an immediate type 1 allergic reaction (52). The molecules of a contact urticant react with specific IgE molecules attached to mast-cell membranes. The cutaneous symptoms are elicited by vasoactive substances, mainly histamine, released from mast cells. Other mediators of inflammation may influence the degree of response. Immunological contact urticaria reaction can extend beyond the contact site and generalized urticaria may be accompanied by other symptoms, such as rhinitis, conjunctivitis, asthma, and even anaphylactic shock. The term ''contact urticaria syndrome'' was therefore suggested by Maibach and Johnson (55). Fortunately, the appearance of systemic symptoms is rare, but it may be seen in cases of strong hypersensitivity or in a widespread exposure and abundant percutaneous absorption of an allergen.

## GUINEA PIG EAR SWELLING TEST

Predictive assays for evaluating the ability of materials to produce nonimmunological contact urticaria have been developed. Lahti and Maibach (57) developed an assay in guinea pigs using materials known to produce urticaria in humans. One-tenth of a milliliter of the material (or control solvent) is applied to one ear of the animal. Ear thickness is measured before application and then every 15 min for 1 or 2 h after application. The maximum response is a 100% increase in ear thickness (within 50 min after application).

Materials can also be screened for nonimmunological contact urticaria in humans. A small amount of the test material is applied to a marked site on the forehead and the vehicle is applied to a parallel site. The areas are evaluated at about 20 to 39 min after application for erythema and/or edema (52).

Differentiation between nonspecific irritant reactions and contact urticaria may be difficult. Strong irritants (e.g., hydrochloric acid, lactic acid, and phenol), can cause clear-cut immediate whealing if the concentration is high enough, but the reactions do not usually fade away quickly. Instead, they are followed by signs of irritation (erythema, scaling, or crusting) 24 h later. Some substances have only irritant properties (e.g., benzoic acid and nicotinic acid esters), some are pure irritants (e.g., SLS), and some have both these features [e.g., dimethyl sulfoxide (DMSO) and formaldehyde].

## TRIMELLITIC ANHYDRIDE-SENSITIVE MOUSE ASSAY

The respiratory allergen, trimellitic anhydride (TMA), has been shown to induce IgE production and immediate ear swelling in mice sensitized to it (58). These authors showed that TMA-sensitized mice have a biphasic ear-swelling response with early (30 min–2 h) and late (24–48 h) phases after topical application of TMA. It was concluded that the first swelling was due to either immediate-type immunological processes or NICU, and the second swelling was due to contact hypersensitivity (i.e., allergic contact dermatitis). This relatively simple method could possibly be a useful tool to study the pharmacology of CU. However, further validation of this model is still required.

## SUBJECTIVE IRRITATION AND PARESTHESIA

Cutaneous application of some chemicals elicits sensory discomfort—tingling and burning without visible inflammation. This noninflammatory painful response has been termed subjective irritation (59). Materials reported to produce subjective irritation include DMSO, salicylic acid, amyl–dimethyl-p-amino benzoic acid and 2-ethoxy ethyl-p-methoxy cinnamate, which are ingredients of cosmetics and over-the-counter drugs. Pyrethroids, a group of broad-spectrum insecticides, produce a similar condition that may lead to temporary numbness, which has been called paresthesia (60). Only a portion of the human population seems to develop nonpyrethroid subjective irritation. For example, only 20% of subjects exposed to 5% aqueous lactic acid in a hot, humid environment developed stinging response (59). Prior skin damage (e.g., sunburn, pretreatment with surfactants, and tape stripping) increases the intensity of responses in stingers. Recent data show that stingers develop stronger reactions to materials causing nonimmunological contact urticaria. The mechanisms by which materials produce subjective irritation have not been extensively investigated. Pyrethroids directly act on the axon, interfering with the channel-gating mechanism and impulse firing (61). It has been suggested that agents causing subjective irritation act via a similar mechanism because no visible inflammation is present.

An animal model was developed to rate paresthesia to pyrethroids and may be useful for other agents (60). Both flanks of 300 to 450 g guinea pigs are shaved and 100 μL of the test material (or vehicle) is spread over approximately 30 $mm^2$ on separate flanks. The animal's behavior is monitored by an unmanned video camera for 5 min at 0.5, 1, 2, 4, and 6 h after application. Subsequently, the film is analyzed for the number of full turns of the head made, usually accompanied by attempted licking and biting of the application sites. Using this technique, it was possible to rank pyrethroids for their ability to produce paresthesia and corresponded to the ranking available from human exposure.

## HUMAN ASSAY

As originally published, the human subjective irritation assay required the use of a 110°F environmental chamber with 80% relative humidity (59). Sweat was removed from the nasolabial fold and cheek, then a 5% aqueous solution of lactic acid was briskly rubbed over the area. Those who reported stinging for 3 to 5 min within the first 15 min were designated as stingers and were used for subsequent tests. Subjects were asked to evaluate the degree of stinging as 0 = no stinging; 1 = slight stinging; 2 = moderate stinging; 3 = severe stinging.

## REFERENCES


1. Bartek MJ, LaBudde JA. Percutaneous absorption in vitro. In: Maibach HI, ed. Animal Models in Dermatology. New York: Churchill–Livingstone, 1975:103–120.
2. Bartek MJ, LaBudde JA, Maibach HI. Skin permeability in vivo: comparison in rat, rabbit, pig and man. J Invest Dermatol 1972; 58:114–123.
3. Simpson WL, Cramer W. Fluorescence studies: carcinogens in skin. Cancer Res 1943; 3:362–369.
4. Dugard PJ. Skin permeability theory in relation to measurements of percutaneous absorption in toxicology. In: Marzulli FN, Maibach HI, eds. Dermatotoxicology, 2nd ed. New York: Hemisphere, 1983:91–116.
5. Scheuplein RJ. Permeability of skin: a review of major concepts. Curr Probl Dermatol 1978; 7:58–68.
6. Scheuplein RJ, Bronough RL. Percutaneous absorption. In: Goldsmith LA, ed. Biochemistry and Physiology of the Skin. New York: Oxford Press, 1983:1255–1295.
7. Feldmann RJ, Maibach HI. Regional variation in percutaneous penetration of [$^{14}$C]cortisone in man. J Invest Dermatol 1967; 48:181–183.
8. Wester RC, Maibach HI. Cutaneous pharmacokinetics: 10 steps to percutaneous absorption. Drug Metab Rev 1983; 14:169–205.
9. Frosch PJ. Irritancy of soap and detergent bars. In: Frost P, Horwitz SN, eds. Principles of Cosmetics for the Dermatologist. St. Louis: CV Mosby, 1982:5–12.
10. Ostrenga J, Steinmetz C, Poulsen B, Yett S. Significance of vehicle composition. II. Prediction of optimal vehicle composition. J Pharm Sci 1971; 60:1180–1183.
11. Noonan PK, Wester RC. Cutaneous biotransformations and some pharmacological and toxicological implications. In: Marzulli FN, Maibach HI, eds. Dermatotoxicology, 2nd ed. New York: Hemisphere, 1983:71–90.
12. Hotchkiss SAM. Skin as a xenobiotic metabolising organ. In: Gibson GG, ed. Progress in Drug Metabolism. Vol. 13. London: Taylor and Francis, 1992:217–262.
13. Anderson C, Sundberg K, Groth O. Animal model for assessment of skin irritancy. Contact Derm 1986; 15:143–151.
14. Franz TJ. Percutaneous absorption. On the relevance of in vitro data. J Invest Dermatol 1975; 64:190–195.

15. Bronaugh, Maibach HI. In Vitro Percutaneous Absorption. Boca Raton: CRC Press, 1992.
16. Jadassohn J. Zur Kenntniss der medicamentosen Dermatosen. Verh Dtch Dermatol Ges 5 Congress 1896:103–129.
17. Bloch B, Steiner–Wourlisch A. Die Sensibilisierung des Meerschweinchens gegen Primeln. Arch Dermatol Syph 1930; 162:349–378.
18. Landsteiner K, Jacobs J. Studies on the sensitization of animals with simple chemical compounds. II. J Exp Med 1936; 64:625–629.
19. Landsteiner K, Jacobs J. Studies on the sensitization of animals with simple chemical compounds. J Exp Med 1935; 61:643–648.
20. Landsteiner K, Chase MW. Studies on the sensitization of animals with simple chemical compounds. IV. Anaphylaxis induced by picryl chloride and 2:4 dinitrochlorobenzene. J Exp Med 1937; 66:337–351.
21. von Blomberg BME, Bruynzeel DP, Scheper RJ. Advances in mechanisms of allergic contact dermatitis: In vitro and in vivo research. In: Marzulli FN, Maibach HI, eds. Dermatotoxicology, 4th ed. New York: Hemisphere, 1991:255–362.
22. Magee PS, Hostynek JJ, Maibach HI. A classification model for allergic contact dermatitis. Quant Struct Activity Relationships 1994; 13:22–33.
23. Code of Federal Regulations. Office of the Federal Registrar, National Archives of Records Service. General Services Administration 1985: Title 16, parts 1500.50—1500.41.
24. Environmental Protection Agency. Pesticides registrations: proposed data requirements. Sec. 158. 135: toxicology data requirements. Fed Reg 1982; 47:53192.
25. Andersen KE, Maibach HI. Multiple-application delayed-onset contact urticaria: possible relation to certain unusual formalin and textile reactions. Contact Derm 1983; 10:227–234.
26. Draize JH, Woodard G, Calvery HO. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membrane. J Pharmacol Exp Ther 1944; 82:377–390.
27. Klecak G. Identification of contact allergens: Predictive tests in animals. In: Marzulli FN, Maibach HI, eds. Dermatotoxicology, 2nd ed. New York: Hemisphere, 1983: 193–236.
28. Kero M, Hannuksela M. Guinea pig maximization test, open epicutaneous test and chamber test in induction of delayed contact hypersensitivity. Contact Derm 1980; 6:341–344.
29. Buehler EV. A new method for detecting potential sensitizers using the guinea pig. Toxicol Appl Pharmacol 1964; 6:341.
30. Klecak G. The Freund's Complete Adjuvant test and the open epicutaneous test. In: Maibach HI, Anderson KE, eds. Contact Allergy, Predictive Tests in Guinea Pigs. Basel: Karger, 1985:152–171.
31. Kaminsky M, Szivos MM, Brown KR. Application of the Hill Top Patch Test Chamber to dermal irritancy testing in the albino rabbit. J Toxicol Cutan Ocular Toxicol 1986; 5(2):81–87.
32. Frosch PJ, Kligman AM. The Duhring chamber: An improved technique for epicutaneous testing of irritant and allergic reactions. Contact Derm 1979; 5:73.

33. Henderson CR, Riley EC. Certain statistical considerations in patch testing. Invest Dermatol 1945; 6:227–230.
34. Draize JH. Procedures for the appraisal of the toxicity of chemicals in foods, drugs, and cosmetics. VIII. Dermal toxicity. Food Drug Cosmet Law J 1955; 10: 722–731.
35. Marzulli FN, Maibach HI. Antimicrobials: experimental contact sensitization in man. J Soc Cosmet Chem 1973; 24:399–421.
36. Marzulli FN, Maibach HI. Use of graded concentrations in studying skin sensitizers: experimental contact sensitization in man. Food Cosmet Toxicol 1974; 12:219–227.
37. Prottey C. The molecular basis of skin irritation. In: Breuer MM, ed. Cosmetic Science, Vol. 1. London: Academic Press, 1978:275–349.
38. Shelanski HA. Experience with and considerations of the human patch test method. J Soc Cosmet Chem 1951; 2:324–331.
39. Irritation: In Vitro Approaches. In: Rouger A, Goldberg L, Maibach H, eds. In Vitro Toxicology. London: Academic Press, 1994:23–185.
40. Motoyoshi K, Toyoshima Y, Sato M, Yoshimura M. Comparative studies on the irritancy of oils and synthetic perfumes to the skin of rabbit, guinea pig, rat, miniature swine, and man. Cosmet Toiletr 1979; 94:41–42.
41. Guillot JP, Gopnnet JF, Clement C, Caillard L, Truhauf R. Evaluation of the cutaneous–irritation potential of compounds. Food Chem Toxicol 1982; 20:563–572.
42. Phillips L, Steinberg M, Maibach HI, Akers WA. A comparison of rabbit and human skin response to certain irritants. Toxicol Appl Pharmacol 1972; 21:369–382.
43. Magnusson B, Hersle K. Patch test methods. I. A comparative study of six different types of patch tests. Acta Dermatol 1965; 45:123–128.
44. Mathias CGT, Maibach HI. Dermatoxicology monographs. I. Cutaneous irritation: factors influencing the response to irritants. Clin Toxicol 1978; 13:333–346.
45. Kligman AM, Wooding WM. A method for the measurement and evaluation of irritants on human skin. J Invest Dermatol 1967; 49:78–94.
46. 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 Sciences. The Toilet Goods Association. Washington DC, 1968:135–145.
47. Rapaport M, Anderson D, Pierce U. Performance of the 21 day patch test in civilian populations. J Toxicol Cutan Ocular Toxicol 1978; 1:109–115.
48. Emery BE, Edwards LD. The pharmacology of soaps. II. The irritant action of soaps on human skin. J Am Pharm Assoc 1940; 29:251–254.
49. Maurer T, Thomann P, Weirich EG, Hess R. The optimization test in the guinea pig. A method for the predictive evaluation of the contact allergenicity of chemicals. Agents Actions 1975; 5:174–179.
50. Kligman AM. A biological brief on percutaneous absorption. Drug Dev Ind Pharm 1983; 19:521–560.
51. Weigand DA, Gaylor JR. Irritant reaction in negro and caucasian skin. South Med J 1976; 67:548–551.
52. von Krogh C, Maibach HI. The contact urticaria syndrome. Semin Dermatol 1982; 1:59–66.
53. Lahti A. Nonimmunologic contact urticaria. Acta Derm Venereol (Stockh) 1980; 60(suppl):1–49.

54. Maibach HI, Johnson HL. Contact urticaria syndrome. Contact urticaria to diethyltoluamide (immediate–type hypersensitivity). Arch Dermatol 1975; 111:726–730.
55. Lahti A, Maibach HI. Species specificity of non-immunologic contact urticaria: guinea pig, rat and mouse. J Am Acad Dermatol 1985; 13:66–69.
56. Lahti A, Maibach HI. An animal model for non-immunologic contact urticaria. Toxicol Appl Pharmacol 1984; 76:219–224.
57. Frosch PJ, Kligman AM. A method for appraising the stinging capacity of topically applied substances. J Soc Cosmet Chem 1977; 28:197–207.
58. Lauerma AI, Fenn B, Maibach HI. Trimellitic anhydride-sensitive mouse as an animal model for contact urticaria. J Appl Toxicol 1997; 17(6):357–360.
59. Cagen SZ, Malloy LA, Parker CM, Gardiner TH, van Gelder CA, Jud VA. Pyrethroid mediated skin sensory stimulation characterized by a new behavioral paradigm. Toxicol Appl Pharmacol 1984; 76:270–279.
60. Vivjeberg HP, VandenBercken J. Frequency dependent effects of the pyrethroid insecticide decamethrin in frog myelinated nerve fibers. Eur J Pharmacol 1979; 58: 501–504.