JOURNAL OF **Pharmaceutical Sciences**

January 1968 volume 57, number 1

Review Article

Topical Toxicity and Testing

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CONTENTS

THE PHARMACEUTICAL or cosmetic chemist, involved with the problems of topical formulations, is often concerned with the potential of his product for skin irritation and sensitivity and confused by the number of possible local toxicity tests. This review is an attempt to outline the nature of the topical toxicities and the principal test methods for irritation and sensitivity. The vital problems of systemic toxicities, such as acute and subacute studies, as well as special tests (*e.g.*, teratogenesis) are not the province of this review.

INCIDENCE OF SKIN REACTIONS

Considering the widespread use of toiletries and cosmetics the incidence of skin reactions is remarkably low (1–4). This is primarily due to the efforts of reputable manufacturers to detect potential hazards before a product is marketed and thus minimize the possibility of adverse side reactions in normal use. The ultimate goal of toxicity studies is to insure safety or harmlessness under the proposed use conditions (5). Calculated risk is sometimes necessary. This must be based on knowledge of compound sensitivities, such as the use of bromoacid dyes in lipsticks or certain resins in nail polishes. The potential toxicities of cosmetics and toiletries have been discussed in several excellent reviews (3, 6–12).

Statistics on the incidence of skin reactions from cosmetics and toiletries are mainly derived from studies by dermatologists on clinical patients (2, 6, 7, 13, 14). Yet dermatitic skin exhibits about 10–50 times the reaction incidence of intact skin (15, 16). Complaint letters to manufacturers vary from 0.2–1.2 per 100,000 units sold (2, 17, 18), while dermatologists report 2–4% reactions (3). It must be remembered that the data on complaint letters assume each unit sold represents a separate user. This is a fallacy since one consumer may actually use multiple packages (19), which would raise the complaints per user. The most valid procedure is to conduct consumer tests in which the product is placed with a relatively large number of users, and each subject is questioned as to irritation side effects as well as product performance.

Judgments of safety and size of human test panels are based on the prediction of 95% certainty of safety. There has been an assumption that the results with 200 test subjects will apply

Received from the Pharmaceutical Research and Development Department, Hoffmann-LaRoche, Inc., Nutley, NJ 07110

to the population at large (20). Yet if 200 subjects contained no reactors, this would denote 95% confidence that not more than 1.5% of the general public will become sensitized. Even in the absence of an adverse reaction on 30,000 persons tested, it is 95% certain that 1 in 10,000 would still be liable to skin reactions (21, 22). Thus, if there are 10 million users, 1,000 cases of dermatitis will probably arise. The prediction of 99.9% safety in a mass population would require an impossibly huge number of subjects (23). The degree and type of use is the cardinal factor. A 0.5% frequency of sensitization (1:200) might be tolerated if the material is in limited distribution and its efficacy is great. However, in a mass distribution cosmetic, 1 in 200 reactions would necessitate further prerelease studies. An incidence of 1 or less in 100,000 is the desirable goal for mass distributed products.

Primary irritation is rather unusual in a modern commercially available product, although new agents are of concern. Agents capable of inducing skin reactions in 1 in 100 or even 1 in 1,000 subjects are readily detected, but it becomes increasingly difficult to predict the irritation or sensitization potential of a preparation causing skin reactions in 1 in 10,000 or even 1 in 100,000. (This is particularly true for topical agents having weak photosensitizing properties.) It is this particular type of agent that concerns those who are responsible for passing on the safety of products that are used by millions of people.

TOPICAL TESTING

No ideal single test procedure exists which will adequately measure irritation and sensitivity potential. There is rather a spectrum of tests to be selected by the trained toxicologist who can secure data and translate it into probable effects in man, and balance these effects against the intended use and possible misuse of the product.

Simpler and less expensive tests are first performed to determine the range of the problems. Usually, animal eye and primary skin irritation tests must first prove favorable before more complex tests are undertaken. The further experimental design should be geared to indicate the possible type injury, the extent or seriousness of the injury, and the margin of safety that prevails under the most extreme conditions of use (24). Yet, even with these considerations it must be understood that tests which are absolutely predictive are, for the present, beyond practical accomplishment (20).

While opinions concerning the value of animal studies vary (25–27), no responsible person would dispense with animal skin tests. If properly judged, on the basis of degrees of variability and not on a "yes–no" basis, animal tests help predict dangers of severe human irritation and, to some degree, the concentrations that may safely be tolerated by man. The tests have their greatest value as comparative tools. The data, with adequate controls, are related to other substances whose hazards have been defined by time and experience. In addition, toxic doses provide an estimate of the margin of safety under normal usage (24). Additional animal studies may be necessary even after a product has been introduced into clinical usage. As the major effects of a preparation are characterized by studying it in detail in man, a more suitable laboratory model, to simulate more closely these actions in man, may be sought by rescreening a wide variety of animals.

Argument continues as to the great differences between human and animal skin. No one can doubt this. Yet it appears more profitable to be concerned with what, if any, correlations can be obtained between animal testing and human clinical experiences, than to be concerned with differences. The mounting costs of clinical tests virtually dictate initial animal tests as an aid in screening and predicting toxicity potential.

Whenever possible, the intended marketing product and package should be studied. If feasible, the program should include parallel studies of a similar product of known safety. If any question of an individual component's safety or "newness" is involved, it should be examined separately. Ultimately, tests must be performed on man under conditions of actual use. Kennon has discussed many of these usage tests in a recent review of toiletry technology (28).

IRRITATION REACTIONS

Primary irritants are substances which damage skin by direct cytotoxic action (134). Reactions are divided according to severity. A strong irritant has been defined as one which will cause 100% of subjects to react in 24 hr. at a definite reasonable concentration (29).

Mild primary irritants exert their reactions after repeated exposure or overexposure. These include soaps, detergents, and solvents that also present a more serious problem of possible sensitization. Mild irritation may become chronic or cumulative after a number of exposures resulting in "skin fatigue." Conversely, after daily exposure to mild irritants, the irritated skin often adapts to these changes, and recovers from and

becomes resistant to further irritation, resulting in "skin hardening" or "skin accommodation" (132).

Generally speaking, agents that cause primary irritation are not selective in their action and will ultimately affect any normal skin if allowed to act, in sufficient concentration, for a long enough period of time. A precise frame of reference is essential for valid assessments of safety. Unfortunately, there is no universal statement that can be made about the type of chemical which will irritate healthy skin. While the pH of normal skin is mildly acid, there is little reason to believe that mildly alkaline products are irritating to the skin. Excellent tests (30) have demonstrated that except for relatively high alkaline or acid materials, pH *per se* is an unreliable criterion for predicting irritancy to skin. Substances which are easily oxidized or reduced are usually more likely to irritate; *e.g.*, hair dyes with strong reducing agents or depilatories containing sulfides. The products most commonly associated with reactions of the primary irritant type are antiperspirants, depilatories, and permanent wave preparations. Many liquid "automatic" or "roll on" mascaras and an occasional cream-type mascara produce false positive primary irritant reactions when patch tested under occlusive coverings. These misleading irritant reactions are apparently caused by solvents and can be avoided by using nonocclusive patch tests (31).

IRRITATION TESTING

Eye Irritation—There are few materials that are unlikely to enter the eye accidentally. Consequently, investigation of animal eye irritancy is the starting point of virtually all safety evaluation programs. The most widely used procedure has been and is the "Draize" test and its modifications (32–53), intended to provide a measure of the irritancy of a product by exposing rabbits under conditions which would resemble an accidental human exposure, such as splashing a substance into the eye. Many descriptions, modifications, and critiques of the test have been published (26, 32, 53–60).

The Draize test (32) scores the effects on the cornea, iris, and conjunctiva of rinsed and unrinsed material after 24, 48, and 72 hr., and 4 and 7 days. The emphasis is on corneal and iris lesions. Eyes that do not clear by the seventh day are considered to have contained severe irritants. For substances covered under the Federal Hazardous Substances Act, a modified procedure has been proposed (61). To aid in interpretation, a colored set of photos are available from FDA (62).

Variabilities in Draize results may stem from personal error, sample size, time of release of an irritant from a formulation, or sample drainage (54). The most viscous samples tend to remain in the eye longer and are more likely to localize in one area, increasing the possibility of irritation. The competence of the trained observer is always the most important factor. While there is considerable variability between laboratories testing the same sample by the "same" Draize techniques (26, 60), the tests have served quite well in delineating moderate and severe irritants. Their main value may lie in comparing an unknown material with control substances whose potential for producing human eye injuries is known through experience. As with other tests, problems arise with nonirritating or mildly irritating substances, or comparison of related formulations in product development.

Variant scoring systems of the Draize test and modifications have yielded greater reliability for comparisons between products. Kay and Calandra (55) score the extent of irritation, its persistency, and the over-all consistency of the data. Test materials are given tentative ratings on the basis of the scores obtained from all 3 tissues (cornea, iris, and conjunctiva) within the first 96 hr. These tentative ratings are adjusted according to the mean total scores at various specified times. Gaunt and Harper (63) judge the conjunctival reaction that is accompanied by persistent and severe injury to the cornea and iris persisting for more than 5 days. The arbitrary scoring system ranges from 1 for a nonirritant to 5 for a severe irritant. S. P. Battista (54) varies exposure conditions to the irritant quantitatively between no-effect and maximum-effect levels. Interpretation and comparison of data are reduced to a single number which represents a time measurement for achieving a level of response. In addition, it enables an estimate of the maximum exposure time that will be tolerated for a given irritant. Time–response curves have been plotted to estimate the time required for a product to produce a given degree of opacity, iritis, or conjunctivitis. When the procedure is repeated with different sample concentrations, a comparative formulation study can have significance.

Several investigators have noted difficulty in correlating rabbit eye irritation with the experience found in humans (2, 26, 57, 60, 64). The rabbit eye is considered more sensitive than that of man. Nevertheless, Rieger and G. W. Battista (26), in their review of the correlation be-

tween animal and human tests, have cited the example of the hair neutralizer which was indicated to be safe according to the rabbit eye test, but which proved to be irritating in human usage.

Study of records of eye accidents in a liquid detergent factory provide a check against the predictive value of the standard rabbit eye irritation tests (21). The animal and human sources were compared on the basis of the number of days required for the eyes to recover completely. Irritation was more severe in the rabbit eyes than in the eyes of the factory workers subjected to actual accidental exposure. In rabbits there was a high incidence of corneal opacity, with some eyes requiring up to 91 days to recover. The effects in humans were limited to conjunctivitis, and all cases recovered within 2 days. The degree and type of irritation produced in the Rhesus monkey eye appeared to more closely approximate that which resulted from exposure of the workers' eyes. Buehler and Newmann (56) studied the response of rabbit and monkey eyes to surfactant solutions and recommend use of the monkey for eye irritancy tests.

In testing some shampoos consideration must be given to the nature of the active ingredients. They may act as anesthetics when placed in the eye. This is dangerous since the user may not be aware that the shampoo has entered the eye and damage may result before it is washed out. To test for anesthetic effects the guinea pig blinking test is used (65).

Aerosol sprays represent a difficult area in which there is lack of standardization for eye tests. Some laboratories apply sprays directly into the rabbit eye. The direct spray may cause eye damage due to the physical impingement of the particles as well as the cooling due to the propellant. This response may mask the eye damage which may be due to the active ingredients. Other workers allow the propellant to evaporate and instill the residue in the eye. This removes the possibility of damage that may be produced by pressure or chilling and allows the experimenter to test only the active ingredients.

The eye irritation test has become so important that further development of a prospective product with excellent sales potential is often arrested purely on the basis of conclusions drawn from eye irritation studies. In fact, some cosmetic firms use ophthalmic irritancy routinely as *the* principal evaluation criterion for checking all new products early in their developmental stages. Products should not be rejected solely on the basis of a questionable Draize, or any other eye test, unless gross injury is detected. The question is to resolve irritancy *versus* possible injury (59). If the product is desirable on a market basis, it would be well to try another species of animal, such as the dog or monkey. If the one or two other species show positive reactions, it is likely that human eye irritation would result.

Skin Irritation—Methods devised to measure skin irritancy are adequate for screening out stronger and moderate irritants, but are virtually insensitive in the low irritancy range. Materials are either placed in single contact with human or animal skin for varied time periods, at varied concentrations, or repeatedly used. These tests determine not only primary irritation but also "skin fatigue," when the skin reacts due to a succession of insults of a subthreshold intensity.

The skins of the mouse, rat, guinea pig, rabbit, miniature pig, and sheep have been used to screen compounds for primary irritant activity. Histologically, their skins do not closely resemble human skin, but from a responsive point of view, they all show changes when irritated. The rabbit and guinea pig are most frequently chosen (66). Materials which cause simple primary irritation of the skin of rabbits generally can be expected to cause a similar response in humans, but not necessarily in all humans. If appreciable edema occurs in rabbit skin, the material should be suspected as a possible vesicant to human skin.

Draize (32) has also proposed the most widely used skin irritation test. Patch tests are read of suspected irritants on the abraded and intact skin of rabbits. A "primary irritation index" is reached. An index of 0–2 indicates mild irritation potential; 2–6, moderate; and over 6 is rated severe, with 8 a maximum.

Roudabush (67) compared the dermal effects of a large number of diverse organic compounds in the guinea pig and rabbit. The results of primary irritation tests showed that the intact guinea pig skin test is as sensitive as, or more sensitive than, the rabbit skin test in eliciting skin reactions. Levenstein and Wolven (65) claim a close correlation between the response to graded patch tests in the rabbit and observations noted in man.

Carter and Griffith (21) point out the pitfalls in placing sole reliance on animal data for assessing primary irritant hazard. They compared a number of unrelated household products by the Draize rabbit irritation index and human patch tests. The products included toilet soap, a general purpose granular detergent, a light-duty liquid detergent, isopropyl alcohol, and a hair dressing. Agreement between the two methods was poor. The product that gave the least reaction in the

human test, the general purpose granular detergent, gave the most severe reaction on the rabbit, and would be classified as a severe irritant on the basis of the animal data.

The Draize rabbit test (32) or Roudabush guinea pig t[illegible](67) are adequate for screening out stronger ir[illegible]ts but are insensitive in the low irritancy range. To obtain responses to moderate or low irritants, exaggerated conditions of exposure of the skin are required to get an incidence of reaction which may be used as a base for extrapolation. These procedures are comparative to decide whether an irritancy is greater than, equal to, or less than some reference substance. The most practical product reference is one whose irritancy potential has become known through widespread use. The detection of moderate and low irritants usually involves increasing the visual sensitivity of the erythema response by injecting a dye substance, which concentrates in the injured areas as a result of increased vascular permeability. Trypan blue has been most widely used (64, 68–71). Recently, use of sulphan blue has been advocated for greater sensitivity (72, 73).

Finkelstein, Laden, and Miechowski (69) have combined the trypan blue technique with formaldehyde presensitization and occlusive patch testing of varied animal skin to give a fairly sensitive and reliable indicator of the irritancy of agents which fall in the low range of the Draize test. The test, omitting presensitization and dye injection, has been adapted for humans (64). Results of the tests indicate whether it is worthwhile to proceed to sensitivity studies and usage trials with a new formulation. With this technique and all similar procedures, selection of proper control formulation is important.

Kligman (29, 134) has critically discussed quantitative measurement of irritants on human skin. He has attempted to use statistical analysis to find the concentration of agent to reach an irritant response in 50% of subjects (ID_{50}). For strong irritants, this value is read directly from a curve of a percentage of reactors *versus* concentration. For weak irritants, patches are applied to the same site for a minimum of 10 days to reach a cumulative frequency plot which estimates the number of days required to cause 50% of the sampled population to develop a threshold irritant response. By inspecting the curves it is usually possible to judge whether 2 agents differ significantly.

Studies of very minor states of irritation or "mildness" use all available tests (21, 74–77). The procedures of Justice, Travers, and Vinson (76) use human arm immersion (HAI), human patch tests, repeat mouse patch tests (RAP), and water transmission through rat and rabbit excised skin. Both the RAP and HAI tests utilize the principle of eliciting a response by repeated exposures of the same area to the test material. In the HAI test, the quantitative difference between two products lies in the number of exposures necessary to achieve a prescribed level of irritation. In the RAP test, the number of exposures is kept fixed and a quantitative measure of the effect of these exposures is made by histological examinaion of the excised skin. The concentration of the test material can also be varied in the RAP test to obtain additional information about the effect of a product on the skin. The most sensitive test for revealing relatively small differences in mildness between the products appeared to be the HAI. The RAP test served best as a preliminary screening of topical products. Materials showing a significant irritation in the RAP test, at standard testing levels, are not given further consideration for use in products. Should the mildness ratings be favorable, studies are continued, employing the other tests. The water transmission test correlates well with the other mildness tests (120, 121). Opdyke and Burnett (77) immerse guinea pigs in products up to their axillae for 4 hr. on 3 successive days, grading mildness on a 0–10 scale.

SENSITIVITY REACTIONS

Allergic sensitization reactions form by far the largest proportion of toiletry and cosmetic reactions (3). An eczematous allergen has been defined as a substance that is not primarily irritating on first exposure but which, in animals or human beings of appropriate genetic constitution, causes the development of a sensitization. Subsequent contact with concentrations that are not irritating to unexposed or nonsensitized individuals produces a reaction (78). An individual may use a cosmetic for years without reacting and then suddenly acquire an allergenic hypersensitivity to the particular material (79). An antigen–antibody system is required for sensitization reactions.

Cross-sensitization is a special form of sensitization which occurs fairly frequently. People affected are not only sensitive to the substance to which their skin was originally exposed, but also to other substances that are chemically related. The closer the relationship and the longer the contact, the greater the incidence and degree of sensitization. Cross-sensitization may be provoked equally by substances which form a related compound as the result of metabolic changes in

the body (9). Kinmont (80) has compiled a list of varied products which are involved in cross-sensitization. Oxidation hair dye cross-sensitivity is fairly common in sensitive individuals (81, 82).

There is hardly a cosmetic or toiletry product to which someone has not become allergic at some time or other. The most frequent sensitizers are the paraphenylenediamine oxidation type of hair dye and the formaldehyde resin in nail lacquers. Perfumes, lipsticks, and sunscreening agents are also among the more frequent offenders. Artificial colorants, principally the halogenated fluoresceins (eosins) in lipsticks, are probably the most frequent single cause of allergic reactions to cosmetics (83). Paraphenylenediamine hair dyes are undoubtedly potent sensitizers to the majority of the population. Yet considerably fewer than 1% of women who dye their hair develop contact allergy. This is probably explained by the fact that the dye is mainly applied to the hair and not to the scalp. While perfumes are often strong sensitizers, the number of skin reactions is small. Perfumes composed of easily oxidizable aldehydes, phenols, and ketones are more liable to cause reactions than those with the more difficultly oxidizable alcohols and esters. With surface-active agents there is great variance in dermal toxicity. Yet, in general, their order of toxicity is cationic > anionic > nonionic (32, 33).

SENSITIVITY TESTING

Delayed type sensitivity reactions are rarely seen if an agent or product is applied to exposed intact skin. Patch tests in humans and animals and intradermal injections in animals aid in provoking the signs of sensitivity.

Animal testing is useful for preliminary screening before any human sensitization testing. Calnan (84) has reviewed the role of animals in contact sensitivity studies. The guinea pig has proved to be the most useful. The most widely accepted animal technique is that of Landsteiner (85), using the guinea pig. Instead of a patch technique, the material is injected intradermally every other day for 10 applications. Following a 2-week rest a challenging dose is injected and the resulting response is compared to the preceding reactions. Sensitization is present if the challenging dose causes a greater skin response than that evoked by any earlier injection. The major limitation of guinea pig testing is the range of sensitivity. Strong sensitizers may be picked up earlier in the guinea pig than in man, but the weak reactors may not be eliminated. These latter must be studied employing human patch test techniques. While humans can react to 1 p.p.m. of a potent sensitizer, such as dinitrochlorobenzene, it is not normally possible to make guinea pigs react to dilutions greater than 1:25,000 (84). However, with current guinea pig sensitization procedures, one may conclude that if a material produces a significant number of reactions, it is also likely to produce sensitizations in human testing (86, 87). Eczematous changes, similar to those occurring in man, have been produced in guinea pigs by first treating their skin with squalene, sodium lauryl sulfate, sorbitan monolaurate[1] (88), and polysorbate 80[2] (89), all of which stimulate acanthosis.

Patch tests, usually with occlusion, enhance sensitivity testing and are universally used, both with animals and humans (12, 16, 20, 26, 90–95). Human patch tests are the province of the dermatologist. Yet, virtually all products are tested by patch test, so it is important to be aware of the varied tests and their limitations. All predictive procedures are designed around the basic technique of insulting the skin of a group of test subjects one or more times and then, after a lapse of time to permit a reactive state to develop, challenging with another application of the material. Substantially increased reactivity to the challenge is taken as evidence of sensitization.

The Schwartz-Peck (S-P) test (96, 97) is the pioneer "prophetic patch test." New products are tested, using a closed patch, on at least 200 subjects. A control of an old formula with a known record of safety is used. If the new formula shows more reactions than the control, it is deemed unsafe. To secure usage data, a 4-week paired-comparison use test of the cosmetic on the same 200 subjects is recommended before trial sale. Trial sale, the final step by S-P definition, is the sale and use of 5,000–10,000 units in one community. The test has certain inherent defects. False positives may occur, since borderline primary irritants can sometimes produce reactions which can be confused with sensitizations when only a patch test reading is made. More important are the false negatives, which are due to the fact that the single application of a small amount of the product is often inadequate to produce sensitization except in the case of strong allergens. Most of the predictive burden is shifted to the use test, but this part of the procedure is probably numerically inadequate to reveal low reaction rates. Also, some products are used only once weekly, or every 4 to 6 weeks,

[1] Marketed as Span 20 by Atlas Chemical Industries, Wilmington, Del.
[2] Marketed as Tween 80 by Atlas Chemical Industries, Wilmington, Del.

and cannot be evaluated for sensitization in a 4-week use test.

The Traub-Tusing-Spoor (T-T-S) (98) and the Brunner-Smiljanic (B-S) (99) tests are extensions or completions of the S-P to widen usage, increase frequency of applications, and use of a larger area of contact with the test substance. Regardless, all these tests suffer in that trial periods are too short, since many chemicals do not cause sensitization until used for many months.

Draize (100) and Shelanski (101) independently published methods which have come to be known as the "repeated insult" test or the Draize-Shelanski test. The test substance is applied every other day for 10 or 15 exposures to 200 subjects. Two weeks after the last exposure the subjects are challenged at a new site. Draize randomizes the exposure. Shelanski applies the occlusive repetitive applications to the same site to give a higher yield of sensitized subjects but tends to magnify the irritant effects of the test formulation. The resulting so-called "skin fatigue," due to summation of irritations under the test conditions, may make the differentiation between sensitization and irritancy more difficult.

Neither the Draize nor the Shelanski procedures specifies the anatomical site to which the patch should be applied or uses a high enough concentration to allow prediction of sensitization in a small panel of volunteers. Maibach and W. Epstein (93) apply the patches to limbs, since the lymph nodes are crucial to development of allergic sensitization of the contact type. The highest nonirritating concentration is used. With these modifications it was possible to detect the marked sensitization and potential of tetrachlorosalicylanilide (TCSA), which would not be predicted with Draize use concentrations.

A judgment of the value of these tests can be made using agents whose sensitizing capacities have become rather well-defined through extensive use. Kligman (20) evaluated a large series of topical drugs and concluded that the S-P, T-T-S, and Draize procedures were all almost useless. The Shelanski test, while still inadequate, was somewhat better.

The basic problem with all the discussed predictive tests is the attempt at utilizing larger and larger numbers of subjects in lieu of a sensitive method of detection (84). The requirements of large numbers of subjects restricts evaluation to those investigators with access to such populations, so that fewer preparations can receive adequate testing. Furthermore, the same subjects are retested too often, which results in false positive reactions because of conditioned irritability (102). What is needed is to enhance the sensitizing capacity of weak allergens to the point where smaller groups of subjects yield more positive responses.

The judicious use of irritation will improve the sensitivity of any animal or human test. Simultaneous use of mild irritants, the allergen, and occlusion appear to aid penetration and enhance sensitization. These combined techniques are used in the triple-freeze (16) and maximization procedures (20, 91, 103, 133).

The triple-freeze technique consists of irritation of the patch test site by freezing for 3 sec. with dichlorodifluoromethane.[3] Occlusion is maintained for 48 hr. after application of a maximal nonirritating concentration of the test compound. The procedure is repeated an additional two times at 5-day intervals (for three exposures). The challenge or eliciting patch test is applied 10 days after the last freeze. While the triple-freeze method has considerable merit, yielding greater sensitivity results than the previously discussed prophetic methods, it does not give adequate reproducible results (84).

The "maximization" provocative patch test of Kligman (20, 91, 103, 133) was primarily designed to yield allergenicity ratings for individual substances, not complex mixtures, finished products, or formulations. It does not seek to predict the ultimate evidence of sensitization in the consuming public, but rather classifies substances according to the sensitization capabilities they exhibit under an arbitrarily defined set of experimental conditions. Essentially, the test consists of a course of five 48-hr. exposures of an allergen to a single skin site which has been previously inflamed by treatment with aqueous 5% sodium lauryl sulfate (SLS). Chemical irritants, such as SLS or dimethylsulfoxide (DMSO) appeared superior to physical agents such as ultraviolet radiation or Scotch tape. Potential sensitizers are divided into 5 grades ranging from weak to extreme allergenic potential (133). A failure to sensitize a single subject demonstrates almost with certainty that the compound is not a significant sensitizer.

The maximization procedure was used to test a large group of diverse substances including cosmetics, drugs, and industrial contactants. There was good correlation between usage and the maximization test results. Sensitization, within limits, was found to be proportional to the surface concentration of the allergen and not to the total amount of allergen. High concentrations are required for weak allergens. The optimal concen-

[3] Freon 12. E. I. du Pont de Nemours & Co., Wilmington, Del.

tration for the challenge patch testing is the highest nonirritating amount, up to a maximum of 10%. The vehicle was an important factor. Petrolatum was the most generally useful and effective. There is some concern that use of occlusion and other methods of facilitating skin damage, such as use of SLS, may create a false impression of allergenicity (95). There is little danger of this if proper judgment is used and further testing is done. Grief (109) utilized the maximization procedure to study a series of compounds used in fragrance formulation. A panel of 25 adult volunteers was tested with 5% sodium lauryl sulfate for 24 hr., and a 48-hr. occlusive patch was applied with high concentrations of the test material. No sensitization was noted.

Most screening series consist of numerous tests, since the greater the number the greater the likelihood of detecting the allergen. This on the whole renders patch testing expensive, tedious, and time consuming. Patch test screening could be simplified by using mixtures which permit screening for several allergens with a single patch. Mixtures sharply reduce the number of patches required in a screening series without curtailing the number of chemicals screened. E. Epstein (104) has demonstrated the usefulness of patch testing mixtures of topical medicaments, rubber additives, and antiseptic agents. Topical medicaments were most suitable for combination. In general, the irritancy of mixtures seems to be related to the sum of irritancy of their components.

Results of predictive tests, as now constituted, serve as guides rather than absolute criteria of sensitization and irritancy. The tests function best when the test substance is used on a comparative basis against control formulations, with known behavior, in actual usage. Positive reactions do not invariably signify sensitization and negative reactions do not rule it out. False positive reactions may be caused by substances which do not produce dermatitis under normal conditions of use but become primary irritants under closed patch test conditions. This is particularly true of depilatories, permanent wave preparations, shampoos, hair tonics, certain hair preparations, and cosmetics containing volatile solvents (11). These should be tested by the "open" method in which the compound is simply rubbed on a small area of the forearm. False negative reactions may also be encountered when the degree of sensitivity is low and when the area involved by the dermatitis is composed of thin skin, such as the eyelid. If the product in question produces a negative patch test result, it should be retested by actual use at the normal site of application.

Further study of the basic mechanisms of sensitization is required before the tests can be significantly improved. Alterations in current test procedures can increase or decrease the number of reactors but do not help in answering the crucial question of the relationship between sensitization results in laboratory test and in consumer usage. Properly supervised consumer use tests are still required to supplement the laboratory studies. The perfect predictive patch test for allergic sensitization has not been devised. This will ideally involve an *in vitro* test easily employed in the laboratory. Until there is additional information on the pathogenesis of delayed hypersensitivity, this approach is improbable. In the interim, predictive patch testing remains a useful tool. Hopefully, in the years to come, we will learn more about the molecular basis for allergic sensitization. It may then be possible to predict sensitizers on the basis of molecular structure (93).

PHOTOSENSITIVITY REACTIONS

Exposure to light of a particular wavelength will evoke allergic photosensitivity in a predisposed individual (39–51, 105, 106). The phototoxic reactions may be manifested as an exaggerated sunburn or as an eczematous response with pigmentation. The wavelength of light to set off a response closely corresponds to the absorption peak of the offending agent and is referred to as the "action spectrum." Most of the phototoxic materials have an action spectrum in the ultraviolet band between 2800 to 4300 Å. (3, 105). Once an individual becomes photoallergenic the reaction time becomes shorter with subsequent exposures. The eruption usually subsides in a few days leaving no residual pigmentation.

The mechanisms involved in photosensitivity are poorly understood (107). Photosensitizers probably act by virtue of photodynamic action or interaction of light and a photosensitizer in the presence of oxygen to cause a destructive photochemical reaction (39). They also may act as free radical reaction initiators. The free radicals produced can cause damage to the intracellular membrane (108). *In vitro* biochemical and biophysical studies strongly suggest that ultraviolet irradiation of halogenated salicylanilides leads to formation of free radicals.

If topically applied, the penetrability of the sensitizer is of decisive importance (110). A photosensitizer must be imbibed by the Malpighian layer of the skin before hypersensitivity

to light can occur (111). Lipid or aqueous solubility, or both, and the type of vehicle are important factors. The furocoumarins, anthracene, and benzpyrene are fat soluble, penetrate well, and are potent photosensitizers when applied topically (112, 113). Penetration of substances that are water soluble only (eosin, rose bengal, acridine preparations, and sulfanilamide) is slight, as is their phototoxic potentiality after external application (114). A number of drugs taken internally also evoke phototoxicity. The chief offenders are sulfonamides, sulfonylurea, diuretics, phenothiazines, chlorothiazides, tetracyclines, and griseofulvin (42).

The dyes used in lipsticks have been reported to cause an occasional photosensitization (38). Considering the vast number of lipsticks sold, the incidence of cheilitis is very low. An example of photosensitization associated with certain perfume ingredients or essential oils is the so-called Berloque dermatitis usually caused by oil of bergamot, which results in skin pigmentation after exposure to sunlight. The photosensitizing agent in oil of bergamot is not defined, although 5-methoxypsoralen may be responsible (115). In spite of the widespread use of such perfumes, few people have Berloque dermatitis because a number of other circumstances must coincide to produce the pigmentation (41).

The reports of photosensitivity to sunscreen agents would seem paradoxical. Yet, monoglyceryl *p*-aminobenzoate (116) and digalloyl oleate (48) have shown sensitivity effects. In these cases the subject patients were shown to have prior sensitizations to a number of related compounds, or were cross-photosensitized.

The halogenated salicylanilides and bithionol are examples of antibacterial compounds that can produce allergic photocontact reactions. Tetrachlorosalicylanilide, particularly, caused a serious flare-up several years ago in England, and to a lesser extent in the United States, and was withdrawn promptly from the market (118–120). Tribromosalicylanilide has been widely used in soap products for several years without any reported cases of photosensitization until quite recently (49, 121). Bithionol is a bacteriostatic agent related to hexachlorophene. It can produce not only ordinary allergic contact dermatitis but photoallergic reactions as well (80, 121–125). Cross reactions may occur between the polyhalogenated salicylanilides and bithionol. Hexachlorophene itself is apparently only a rare sensitizer.

The major sensitivity activity of the salicylanilides resides in the salicylic acid ring with a carboxyl and hydroxyl group being essential. At least one halogen atom is also required (126). The mechanism appears to be a photoactivation of the salicylanilide ring by 3600 Å. radiation, followed by free radical formation at the 3 or 5 carbon–halogen bond. Ensuing loss of free radical activity is associated with a chemical binding of the free radical (hapten) to protein (complete antigen formation).

PHOTOSENSITIVITY TESTING

Suspected agents are applied as photopatches in the same concentrations as used for ordinary patch tests (50). Tests are particularly indicated if an agent is present which has a chemical structure resembling known photosensitizers, such as chlorpromazine, promethazine HCl,[4] bithionol, tribromosalicylanilide, dibromosalicylanilide, and tetrachlorosalicylanilide.

Natural sunlight is the ideal source for photosensitization testing, but it is usually not regularly available at a given time. Artificial light sources are useful and have been advantageously substituted (35, 43, 46, 105).

Until recently, attempts to produce drug photosensitization in laboratory animals were generally unsuccessful. Guinea pigs (43, 72, 107, 127, 128), rabbits (32), rats (129), and mice (129, 135) have been reasonably satisfactory for some photosensitization substances. In some instances, it has not been possible to elicit photosensitization in animals to substances that regularly produce this response in man.

Vinson and Borselli (127) use stress conditions to elicit skin responses in guinea pigs to marginal sensitizers. If the results of this test and the Landsteiner-Jacobs test are both negative the test material can probably be tested on humans with good assurance that no photosensitization problem will develop. If an agent passes both tests, but shows cross-photosensitivity, additional tests on the preparation in the intended vehicle should be conducted. Wolven and Levenstein (72) used the Vinson-Borselli test, adding intraperitoneal injection of sulphan blue to better visualize the inflamed area and increase the sensitivity of the test. Sams and J. Epstein (107) use guinea pigs to test phototoxicity of systemic drugs. Sunlight, initially used, proved unreliable because the intensity of light varies and because the survival of guinea pigs exposed to sunlight is dependent on the temperate climate. The phototoxic reactions could be produced with either a bank of fluorescent lamps or a mercury vapor lamp (43, 130), but only when Mylar transparent plastic (duPont) was interposed between

[4] Phenergan. Wyeth Laboratories., Philadelphia, Pa.

the light source and the experimental animals. The plastic absorbs all wavelengths below 3100 Å. It prevents normal erythema from developing in control animals but has no effect on the phototoxic response. This represents a means of enhancing the contrast between the control and experimental animals. Using Mylar, phototoxicity of chlorpromazine (43) and demethylchlortetracycline (131) could be demonstrated. Recently, hairless mice have been used in a test which may have predictive phototoxic value, especially for new drugs (129).

The Curwen-Jillson (106) technique is in chief human use to differentiate photoallergic from phototoxic reactions. The ordinary patch test, using the suspected photosensitizer, is first performed in triplicate. Twenty-four hours later a sub MED (minimal erythema dose) is applied to one uncovered test site and a DED (delayed erythema dose), which is 8 times the MED, is applied to the second uncovered test site. The third patch site is a control. A phototoxic reaction is present when there is a sharply demarcated erythema at the sub MED irradiated patch test site. This is usually most marked 24 hr. after irradiation. A photoallergic reaction is present when there is an eczematous or papular response at the DED irradiated test site. This usually appears 48 hr. after irradiation (47).

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Keyphrases

Topical toxicity—review
Skin reactions—incidence
Topical testing
Irritation reactions
Irritation testing
Sensitivity reactions
Sensitivity testing
Photosensitivity reactions
Photosensitivity testing