Current Status of Predictive Animal Models for Drug Photoallergy and Their Correlation With Drug Photoallergy in Humans[1,2]

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ABSTRACT—The number of adverse responses considered to be drug photosensitivity reactions account for only an exceedingly small percentage of the total undesirable effects from environmental chemicals. However, the rising incidence of and severe disability resulting from drug photosensitivity, especially when the photosensitivity is of the persistent light reactor type, indicate that increased photobiologic research and development efforts are required. Predictive tests are an obvious approach to minimize or eliminate those chemicals showing a risk–benefit ratio that is undesirable to society in general or to an unknowing individual in particular. Animal models with predictive value for determining the risk of photoallergic contact dermatitis in humans have undergone considerable modification during the past decade. This study reports an improved experimental guinea pig model for inducing photoallergic contact dermatitis to musk ambrette. In contrast to previously described models that used Freund's adjuvant, this model does not require nuchal stripping with cellophane tape. Control studies for primary irritancy, phototoxicity, allergic contact dermatitis, and "angry back" syndrome were included in the experimental design. Only photoallergic contact dermatitis was observed. Although the technique used to demonstrate this phenomenon is conducive to standardization, additional studies are required to ascertain whether or not other chemicals known to be photoallergic in humans can also be demonstrated with this animal model.—JNCI 1982; 69:237–244

The public in general and the scientific community in particular recognize that the introduction of innumerable new chemicals into our environment has been associated with both benefits and risks. Although these agents have been designed to aid the public, some have clearly entailed hazards of varying degree, as exemplified by hepatotoxic effects of vinyl chloride (*1*) and the oncogenic effects of diethylstilbestrol on vaginal tissue (*2*).

A subset of adverse reactions to environmental chemicals is photosensitivity reactions involving the skin. The term "drug photosensitivity" has been used to describe adverse reactions associated with patients exposed to sunlight after they have received particular medications. Although originally used in this limited clinically therapeutic sense, the term has been expanded in recent years to include reactions to chemicals used in cosmetics (*3*), food preservatives (*4*), household cleaners (*5*), fragrances (*6*), agricultural materials (*7*), and industrial by-products (*8*).

While the incidence of drug photosensitivity reactions has been quite low, it appears to be increasing. There is evidence in the literature of tens of thousands of persons affected with drug photosensitivity reactions during recent decades. In addition, there is a particular type of photosensitivity known as a persistent light reaction (*9*) that can be totally disabling. The increasing incidence and severity of this type of photosensitivity problem have served to illustrate the need for further photobiologic research efforts. It would be particularly desirable to have an animal model that could predict the potential of chemicals or drugs for producing the full range of adverse photosensitivity reactions.

Several models have been described and modifications continue to be developed. This paper is restricted solely to the subjects of development and evaluation of animal models for the induction of photoallergic contact dermatitis and for the assessment of their correlation with clinical events and photosensitivity tests in humans.

GENERAL REVIEW

A number of chemicals have been reported to cause photocontact dermatitis. These reactions are mediated by two broad mechanisms of action, phototoxic and photoallergic pathways. Their salient features are noted in table 1 and text-figure 1.

The mechanism involved in photoallergic contact dermatitis is immunologic and of the delayed hypersensitivity type. The role of light is restricted to photochemically altering the hapten or facilitating its combination with carrier protein (*10*). Following formation of this complete "photoantigen," it is believed that this unit is further processed by macrophages and comes in contact with T-lymphocytes in a manner similar to that of antigens associated with an ordinary delayed hypersensitivity immunologic response (*11*). The complete photoantigen is recognized as foreign on subsequent environmental exposure when sensitized T-lymphocytes are now present. An immunologic response is then initiated which results in erythema in laboratory animals and a papulovesicular, eczematous response in humans (*12*). This hypothesis has been supported by both in vitro and in vivo studies. The following are representative examples:

In Vitro Studies

The photoadducts of TCSA and Jadit (buclosamide) in combination with protein, when assayed with models such

Abbreviations used: TCSA=3,3′,4′,5-tetrachlorosalicylanilide; UVA=UV, 320–400 nm; UVB=UV, 280–320 nm.

[1] Presented at the Seventh Food and Drug Administration Science Symposium ("Photochemical Toxicity: Toxic, Allergic, and Carcinogenic Aspects With Emphasis on Predicting Effects in Humans") held at the Uniformed Services University of the Health Sciences, Bethesda, Md., March 16–17, 1981.

[2] Supported in part by Public Health Service (PHS) grant ES-01041-08 from the National Institute of Environmental Health Sciences, by PHS grant RR-00645 from the Division of Research Resources (National Institutes of Health), and by the Shiseido Co., Ltd., Tokyo, Japan.

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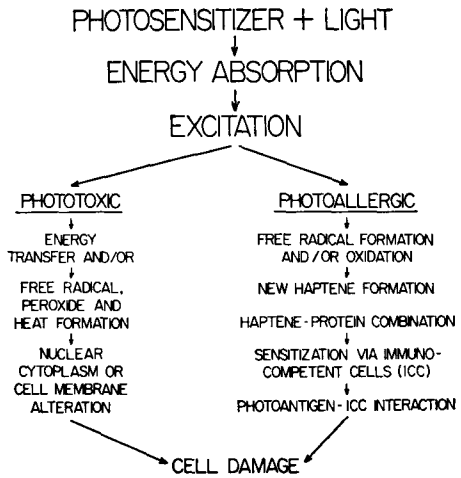
TABLE 1.—*Mechanisms of drug-induced photosensitivity: Comparison of phototoxic and photoallergic reactions in test models*

Mechanism	Reaction	
	Phototoxic	Photoallergic
Incidence	Usually relatively high (theoretically 100%)	Usually lower
Reaction possible on first exposure	Yes	No
Incubation period necessary after first exposure	No	Yes
Persistent light reaction possible	No	Yes
Cross-reactions to structurally related agents	No	Frequent
Passive transfer	No	Possible
Lymphocyte stimulation test	No	Possible
Macrophage migration inhibition test	No	Possible



TEXT-FIGURE 1.—Mechanism of phototoxic and photoallergic reactions.

as the macrophage inhibition and lymphocyte stimulation tests, have demonstrated positive results (*13*, *14*).

In Vivo Studies

Passive transfer of photosensitivity to TCSA with the use of peritoneal mononuclear cells from photosensitized guinea pigs has been demonstrated (*15*, *16*). The most comprehensive data in laboratory animals concerning induction of photoallergy to halogenated salicylanilides and its derivatives were those of Morikawa et al. (*17*). Recent studies by M. Takagawa and Y. Myochi (University of Kyoto, Kyoto, Japan) indicate the feasibility of using inbred strains of mice (BALB/c, DBA/2, C3H, and C57BL) for the induction of photoallergic contact dermatitis to TCSA, tribromsalan, and bithionol.

The first induction of experimental photoallergic contact dermatitis in guinea pigs was reported by Schwarz and Schwarz-Speck (*18*) in 1957. Their demonstration used sulfanilamide as a test model. Following this, Vinson and Borselli (*19*) induced contact photoallergic reactions with TCSA. Their technique has been considerably modified but basically confirmed by several other groups (*20–22*). More recently, contact photoallergies to musk ambrette (*22*, *23*) and 6-methylcoumarin (*24*, *25*) have been induced.

The guinea pig is presently the only experimental animal used to demonstrate photosensitivity of the delayed hypersensitivity type in all of the reported photoimmunologic animal studies. Hartley strain albino guinea pigs (375–425 g) are routinely used. Females appear to be preferable because there is likely to be less scratching of phototest sites.

Table 2 lists the techniques of inducing contact photoallergy in guinea pig models. In all the reported models, after induction and a suitable incubation period, elicitation is accomplished by topical application. These techniques are remarkably similar to those reported for inducing reactions in humans (*26*).

Clinical reports of photosensitivity in humans include reports on chlorpromazine (*27*), bithionol (*28*), Jadit (*29*), and promethazine (*30*). Table 3 presents a comprehensive list of agents reported to induce photoallergic contact dermatitis in humans.

Because of the risk and hazards of injury of test subjects by photosensitization (*31*), the following data in laboratory animals are stressed, indicating our preference for the use of a predictive animal-screening test model for photoallergic contact dermatitis before tests are initiated in humans.

Induction Techniques in Guinea Pigs

The typical induction procedure consists of the topical application of a relatively high concentration (10–100 times more than the lowest eliciting concentration) of the photosensitizer followed by UV irradiation. The entire induction procedure involves solely the nuchal region and is repeated five times during a 7- to 10-day period (table 2). Elicitation of photoallergic contact dermatitis is restricted to the depilated surface of the back.

Induction with UVB irradiation.—This model, introduced by Vinson and Borselli (*19*), has undergone numerous modifications and is an excellent one for inducing photosensitivity to halogenated salicylanilides. It uses both UVA and UVB radiations during the induction phase. As mentioned, although it has undergone extensive modification, this widely established model has been duplicated in several laboratories (*20–22*). However, it is ineffective in inducing photoallergy to 6-methylcoumarin and musk ambrette. A possible hazard involves corneal burns from UVB radiation expo-

TABLE 2.—*Techniques used to increase index of photoallergic contact dermatitis in guinea pigs*

Nuchal technique[a]	Example of photosensitizer
UVB radiation	Halogenated salicylanilides
Sodium lauryl sulfate	Halogenated salicylanilides
Skin "stripping" with cellophane tape	Musk ambrette
Stripping plus intradermal injection of Freund's adjuvant	Musk ambrette 6-Methylcoumarin
Intradermal injection of Freund's adjuvant without stripping	Musk ambrette

[a]These procedures precede irradiation of topically applied chemical with UVA radiation.

TABLE 3.—*Agents reported to induce photoallergic contact dermatitis in humans*

Agent[a]	Suggested concentration,%, for photopatch test
TCSA	0.1
3′,5′-Dibromosalicylanilide	1
4′,5′-Dibromosalicylanilide	1
Bithionol	1
Jadit	1
3,4′,5-Tribromosalicylanilide	1
Promethazine	1
Chlorpromazine	1
Dowicide 32	1
6-Methylcoumarin	5
Musk ambrette	5
Sulfonilamide	1
p-Aminobenzoic acid	1
2-Ethoxyethyl *p*-methoxycinnamate	1
Isoamyl dimethylaminobenzoate	5

[a] Petrolatum was used as a vehicle.

sure; therefore, the investigator's eyes must be protected with goggles.

Induction with sodium lauryl sulfate.—This model, introduced by Horio (*32*), offers promise and should be further evaluated. The induction procedure consists of the topical application of a 20% aqueous solution of sodium lauryl sulfate to the nuchal area. One hour later the photosensitizer is applied to the same site and immediately thereafter the site is exposed to UVA radiation. The procedure is repeated five to ten times during a 10-day period. The technique appears simple and effective. It is reported to produce a high index of photosensitization to halogenated salicylanilides and bithionol. Unfortunately, there are only limited reports of usage by photobiologists.

Induction with skin stripping with cellophane tape.—The induction phase consists of the repeated application of cellophane Scotch tape to the nuchal site until glistening is noted. The photosensitizer is then applied to this area, after which the site receives UVA radiation. The procedure is repeated three to five times during a 7-day period on the nuchal area. The rationale of stripping is presumed to involve increased percutaneous absorption of the photosensitizer, but the effectiveness of the model may also be related to the inflammatory response. This induction procedure appears analogous to the maximization test in humans and was successful for induction of musk ambrette photosensitivity in guinea pigs (*22*). However, in our studies, it was ineffective in inducing photoallergy to 6-methylcoumarin. A disadvantage of the technique is that a thick crust often forms on the nuchal induction site and impedes absorption.

Induction with Freund's complete adjuvant and cellophane stripping.—Freund's complete adjuvant, injected intradermally, has been demonstrated to increase the incidence of photosensitization to musk ambrette and 6-methylcoumarin in the guinea pig (*23*). This technique involves injecting Freund's adjuvant into four sites at the periphery of the depilated nuchal area, stripping with cellophane tape, applying the test substance, and exposing the region to UVA radiation. During the entire photosensitizing procedure, the adjuvant is injected only once, but the remainder of the induction steps are performed a total of five times (fig. 1). After 2 weeks, photosensitivity is assessed after depilation of the back for the first time, application of the test material to the back, and exposure of the back to UVA radiation (fig. 2). These experiments pose the disadvantage of variations in the manner by which and the extent to which cellophane tape stripping are accomplished and they make it difficult for other investigators to reproduce accurately and uniformly, in a controlled manner, the stripping procedures. Accordingly, the following studies were designed to test whether or not stripping was necessary for induction of photoallergic contact photosensitivity to musk ambrette.

MATERIALS AND METHODS

Experimental animals.—Twenty-three Hartley strain female albino guinea pigs weighing 375–450 g were tested. Musk ambrette (2-methoxy-3,5-dinitro-4-methylbutylbenzene) was used as a test agent. The purity of musk ambrette was assayed by high-pressure liquid chromatography and thin-layer chromatography.

Light sources.—Black light fluorescent tubes (General Electric Co.) emitting radiation in the 320- to 400-nm range were employed. The fluence rate measured at 12.5 cm through 3 mm of window glass was 2.85 $mW \cdot cm^{-2}$. All black light irradiation (10.2 $J \cdot cm^{-2}$) is nonerythrogenic in the absence of the photosensitizer.

Induction of photosensitivity in nonstripped nuchal skin.—The nuchal area (2.5×2.5 cm) of 11 guinea pigs was shaved and depilated with Nair, and 0.1 ml of a 10% solution of musk ambrette in acetone was applied. Before this procedure, 0.1 ml Freund's adjuvant (fig. 1) had been injected into four nuchal sites. Five treatments were given during a period of 10–11 days. Each treatment was followed by irradiation with black light fluorescent tubes as previously described.

Induction of photosensitivity in stripped nuchal skin (control).—The nuchal area of 12 guinea pigs was shaved, depilated with Nair, and stripped with cellophane tape for three or four of the five treatments. Freund's adjuvant was injected into four sectors. Musk ambrette (0.1 ml of a 10% solution in acetone) was applied within 15 minutes after stripping. The animals were irradiated with the same doses of light as were used for the nonstripped animals. The sensitization treatment of both the cellophane tape-stripped and nonstripped groups was performed five times during a period of 10–11 days.

Tests for elicitation of contact photosensitivity to musk ambrette.—The guinea pigs were challenged 17–22 days after the last nuchal sensitization exposure. The shaven and depilated lumbar area which had received no previous exposure to musk ambrette or light was demarcated into six sites (2.5×3.5 cm) with masking tape. The animals previously exposed to 10% musk ambrette in their nuchal region were challenged with 10, 1, and 0.1% concentrations of musk ambrette in ethanol on their dorsal lumbar skin (fig. 2). Each concentration (0.1 ml) was applied to symmetrical sites on the left and right sides of the animal. The right side was shielded with light-opaque material. A nonerythrogenic dose (10.2 $J \cdot cm^{-2}$) from black light fluorescent tubes was administered 30 minutes after application of the challenge solutions.

TABLE 4.—*Induction of photoallergic contact dermatitis to musk ambrette with the use of Freund's complete adjuvant, with and without cellophane tape stripping*

Procedure	Concentration of musk ambrette, %	No. of irradiated animals reacting/total No. of animals irradiated[a]			No. of unirradiated animals reacting/total No. of unirradiated animals
		1+	2+	Total No. of irradiated animals reacting/total No. of animals irradiated	
Nonstripping	0.1	4/11	0/11	4/11	0/11
	1.0	5/11	1/11	6/11	0/11
	10	5/11	1/11	6/11	0/11
Stripping	0.1	4/12	2/12	6/12	0/12
	1.0	6/12	4/12	10/12	0/12
	10	6/12	4/12	10/12	0/12

[a] Grading of reaction: 1+=mild erythema without edema; 2+=moderate to strong erythema without edema.

TABLE 5.—*Control studies for photoallergic contact dermatitis to musk ambrette*

Mechanisms evaluated	Chemical[a]	No. of animals	Result
Phototoxicity	100% musk ambrette	20	No primary irritant or phototoxic reaction noted on first exposure
Allergic vs. photoallergic contact dermatitis related to humans	$NiSO_4$	12	Contact allergy but no photocontact allergy induced with Freund's adjuvant
Allergic reactivity unrelated to humans	$CuSO_4$	12	No induction of allergic or photoallergic contact dermatitis
Nonspecific reactivity, angry back syndrome	TCSA, $CuSO_4$, and musk ambrette	12	No reaction to $CuSO_4$ and musk ambrette in animals photosensitized to TCSA and challenged with TCSA, $CuSO_4$, and musk ambrette

Evaluation of test results.—Erythema on the test sites was evaluated at 24 hours after irradiation with the use of a scoring system ranging from 0 to 3. A score of 1 or higher was considered a positive response.

RESULTS AND CONTROLS

Contact photoallergy to musk ambrette.—As noted in table 4, photosensitization to musk ambrette was induced with both the stripping and nonstripping procedures (fig. 3). The incidence of photosensitization and magnitude of response were greater in the stripped group.

Controls of photoallergy (table 5).—So that false-positive photosensitivity responses of a nonspecific nature could be ruled out, attempts were made to photosensitize guinea pigs to nickel sulfate, a known ordinary contact allergen in humans which has induced no cases of photoallergy in humans to date, and to cupric sulfate, a nonallergic agent in humans. Allergic contact but not photoallergic contact dermatitis was induced to nickel sulfate by use of Freund's adjuvant technique (Ichikawa H: Personal communication). Neither allergic nor photoallergic dermatitis was induced with cupric sulfate.

Controls for nonspecific reactivity ("angry back" syndrome).—Animals photosensitized to TCSA were challenged with TCSA and cupric sulfate. Reactions were noted only at TCSA-treated sites.

Control studies of phototoxicity: Phototoxicity to musk ambrette, anthracene, and 8-methoxypsoralen.—As previously described, 20 guinea pigs with no previous nuchal sensitization to musk ambrette were challenged with 50, 20, 10, 5, 3, and 1% concentrations of musk ambrette in acetone (*32*). With the use of radiation identical to that which elicited photoallergy, none of this group showed any visible reaction.

CONCLUSION

This modified experimental model for inducing photoallergic contact dermatitis in guinea pigs demonstrates the successful induction of photoallergic contact dermatitis to musk ambrette. In contrast to previously described models using Freund's adjuvant, this model did not require stripping with cellophane Scotch tape. Additional studies are needed to further demonstrate the correlation of the results seen with this model with events found in humans.

REFERENCES

(*1*) ROBBOY SJ, KAUFMAN RH, PRAT J, et al. Pathologic findings in young women enrolled in the National Cooperative Diethylstilbestrol Adenosis (DESAD) Project. Obstet Gynecol 1979; 53:309–317.

(*2*) PITOT HC, GOLDSWORTHY T, CAMPBELL HA, POLAND A. Quantitative evaluation of the promotion by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin of the hepatocarcinogenesis from diethylnitrosamine. Cancer Res 1980; 40:3616–3620.

(*3*) FISHER AA. Contact dermatitis. In: Contact dermatitis. Philadelphia: Lea & Febiger, 1973:197–214.

(*4*) HARBER LC, LEVINE GM. Photosensitivity dermatitis from household products. General Practitioner 1969; 39:95–100.

(*5*) HARBER LC, HARRIS H, LEIDER M, BAER RL. Berloque (Berlock) dermatitis. Arch Dermatol 1964; 90:572–576.

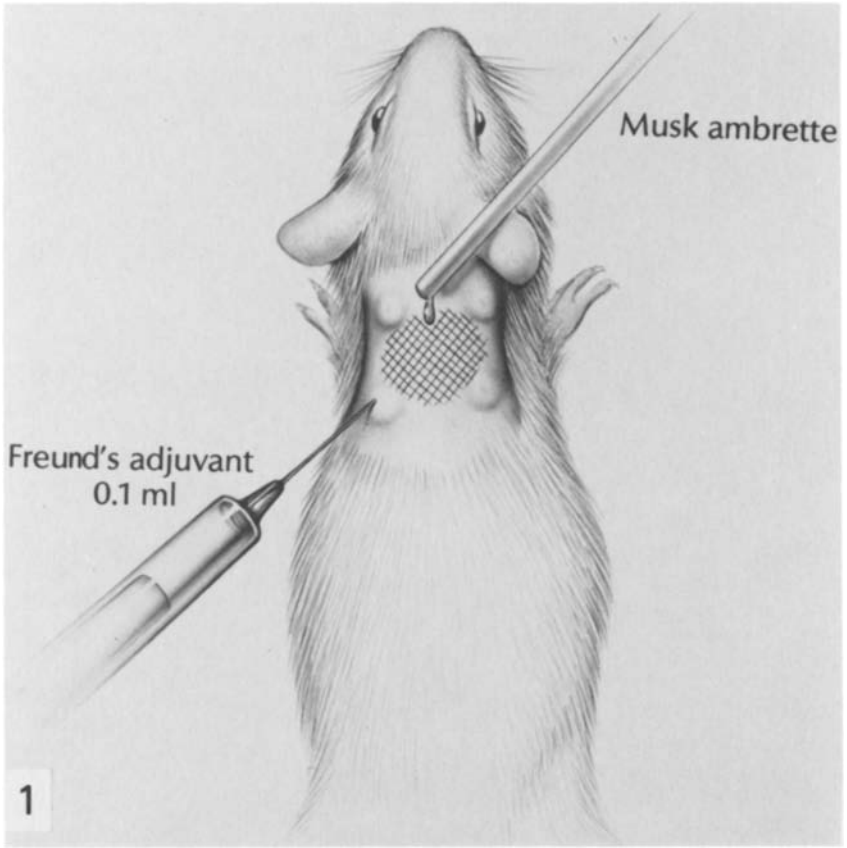
(*6*) HARBER LC, BAER RL. Pathogenic mechanisms of drug-induced photosensitivity. J Invest Dermatol 1972; 58:327–342.

(*7*) EMMETT EA. Phototoxicity from exogenous agents. Photochem Photobiol 1979; 30:429–436.

(*8*) HJORTH N, MOELLER H. Phototoxic textile dermatitis ("bikini dermatitis"). Arch Dermatol 1976; 112:1445–1447.

(*9*) JILLSON OF, BAUGHMAN RD. Contact photodermatitis from bithionol.

Arch Dermatol 1963; 88:409–418.
(10) Herman PS, Sams WM Jr. Requirement for carrier protein in salicylanilide sensitivity: The migration-inhibition test in contact photoallergy. J Lab Clin Med 1971; 77:572–579.
(11) Harber LC, Baer RL, Bickers DR. Techniques of evaluation of phototoxicity and photoallergy in biologic systems, including man, with particular emphasis on immunologic aspects. In: Fitzpatrick TB, Pathak MA, Harber LC, Seiji M, Kukita A, eds. Sunlight and man: Normal and abnormal photobiologic responses. Tokyo: Univ Tokyo Press, 1974:515–528.
(12) Harber LC, Kochevar IE, Shalita AR. Mechanisms of photosensitization to drugs in man. In: Parrish J, ed. Photomedicine. New York: Plenum, 1980:143–144.
(13) Herman PS, Sams WM Jr. Immunologic investigation. In: Soap photodermatitis. Springfield, Ill.: Thomas, 1972:87–89.
(14) Jung EG, Hornke J, Hajdu P. Photoallergic durch 4-Chlor-2-Hydroxy-Benzoesauer-*N*-Butylamid. Arch Klin Exp Dermatol 1968; 233:287–295.
(15) Harber LC, Targovnik SE, Baer RL. Contact photosensitivity to halogenated salicylanilides: In man and guinea pigs. Arch Dermatol 1967; 96:646–656.
(16) Harber LC, Baer RL. Mechanisms of drug photosensitivity reactions. Toxicol Appl Pharmacol 1969; 3:58–67.
(17) Morikawa F, Nakayama Y, Fukuda M, et al. Techniques for evaluation of phototoxicity and photoallergy in laboratory animals and man. In: Fitzpatrick TB, Pathak MA, Harber LC, Seiji M, Kukita A, eds. Sunlight and man: Normal and abnormal photobiologic responses. Tokyo: Univ Tokyo Press, 1974:529–557.
(18) Schwarz K, Schwarz-Speck M. Experimentelle Untersuchungen zur Frage der Photoallergic der Sulfonamide. Dermatologica 1957; 114:232–243.
(19) Vinson LJ, Borselli VF. A guinea pig assay of the photosensitizing potential of topical germicides. J Soc Chem 1966; 17:123–130.
(20) Griffith J, Carter RD. Patterns of photoreactivity and cross reactivity in persons sensitive to TCSA. Toxicol Appl Pharmacol 1968; 12:304–309.
(21) Herman PS, Sams WM Jr. Requirement for carrier protein in salicylanilide sensitivity: The migration inhibition test in contact photoallergy. J Lab Clin Med 1971; 77:572–579.
(22) Kochevar IE, Zalar GL, Einbinder J, Harber LC. Assay of contact photosensitivity to musk ambrette in guinea pigs. J Invest Dermatol 1979; 73:144–146.
(23) Ichikawa H, Armstrong RB, Harber LC. Photoallergic contact dermatitis in guinea pigs: Improved induction technique using Freund's complete adjuvant. J Invest Dermatol 1981; 76:498–501.
(24) Giovinazzo VJ, Harber LC, Armstrong RB. Photoallergic contact dermatitis to musk ambrette, clinical report of two patients with persistent light reactor patterns. J Am Acad Dermatol 1980; 3:384–393.
(25) Harber LC. Current status of mammalian and human models for predicting drug photosensitivity. Soc Invest Dermatol. In press.
(26) Willis I, Kligman AM. The mechanism of photoallergic contact dermatitis. J Invest Dermatol 1968; 51:378–384.
(27) Epstein JH, Brunsting LA. Topical application of chlorpromazine; its effect on the erythema response to ultraviolet light. J Invest Dermatol 1958; 30:91–94.
(28) Jillson OF, Baughman RD. Contact photodermatitis from bithionol. Arch Dermatol 1963; 88:409–418.
(29) Burry JM, Hunter GA. Photocontact dermatitis from Jadit. Br J Dermatol 1970; 82:224–229.
(30) Sidi E, Hincky M, Gervais A. Allergic sensitization and photosensitization to phenergan cream. J Invest Dermatol 1955; 24:345–352.
(31) Willis I, Kligman AM. The mechanism of the persistent light reactor. J Invest Dermatol 1968; 51:385–394.
(32) Horio T. The induction of photocontact sensitivity in guinea pigs without UVB radiation. J Invest Dermatol 1976; 67:591–593.



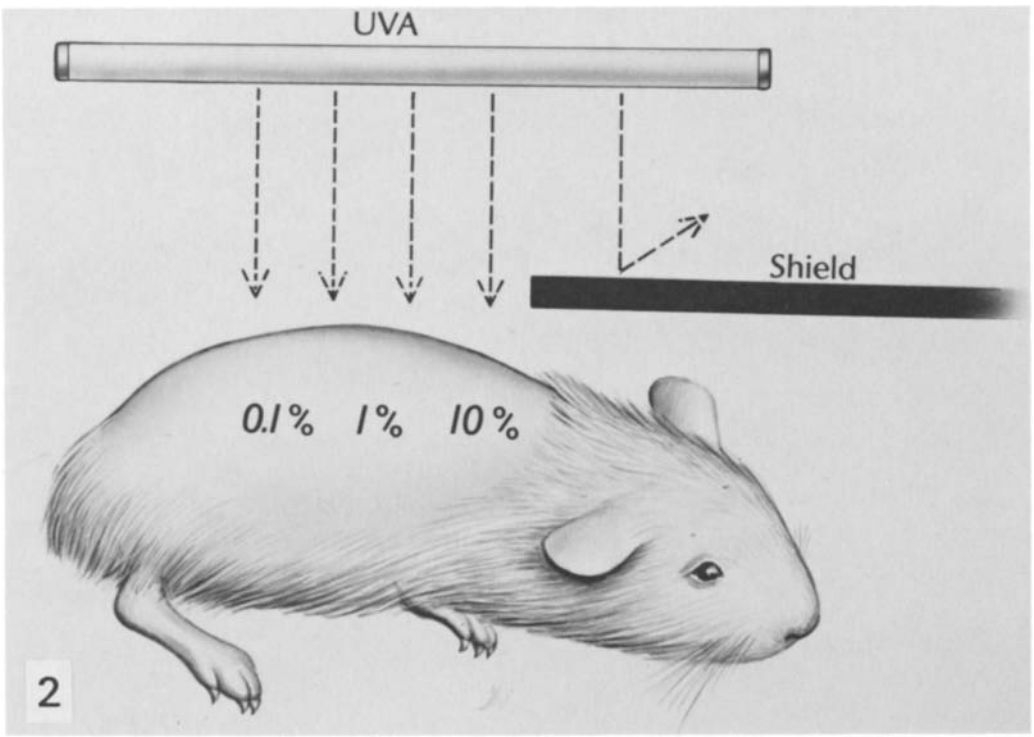


FIGURE 1.—Induction of photosensitivity in the nuchal area with the use of Freund's adjuvant.
FIGURE 2.—Elicitation of photoallergic contact dermatitis to musk ambrette.

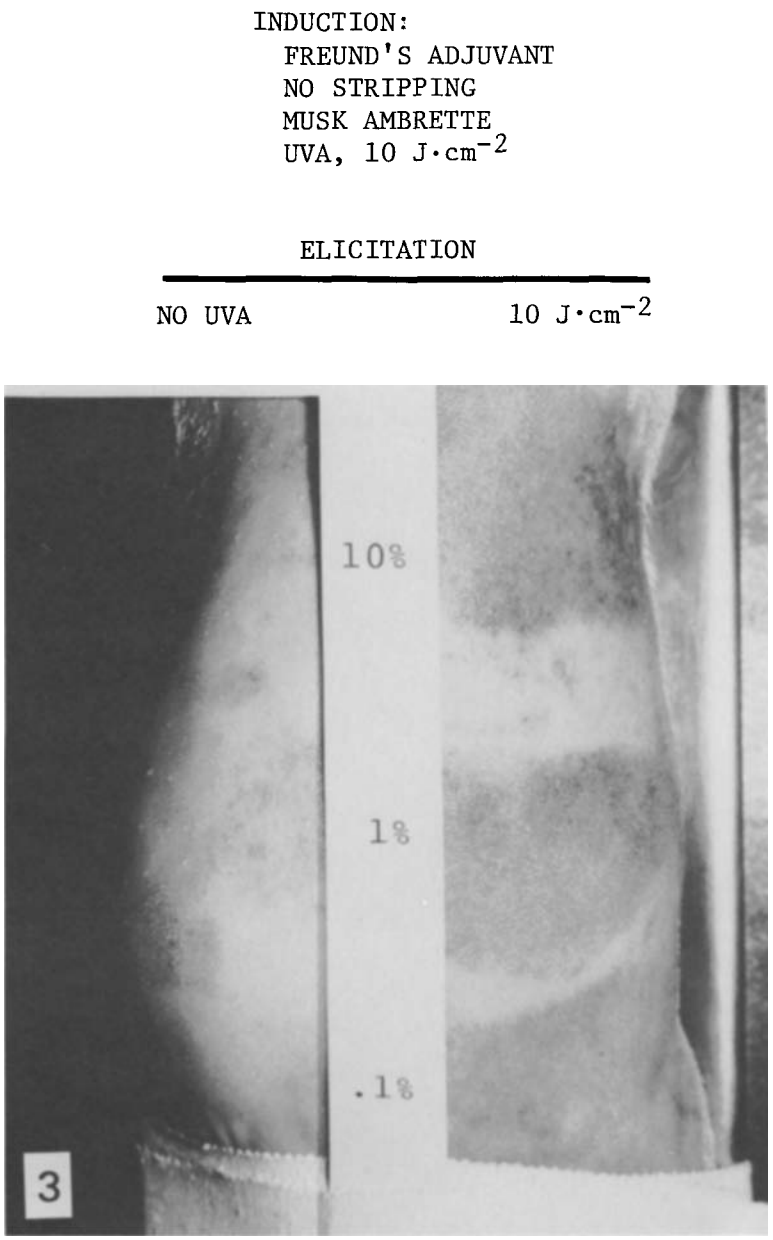


FIGURE 3.—Erythema elicited 24 hr after exposure to 10, 1.0, and 0.1% musk ambrette and UVA radiation. Similar sites that were unirradiated showed no erythema.

DISCUSSION

J. Middleton: I have a question with regard to the control animals used in your studies. I have had considerable experience with the Magnusson guinea pig maximization test, which we use in Europe. In this test, if you inject with adjuvant, you can show that you increase the susceptibility not only to sensitization, but also to irritation.

My question is, in the studies you showed us, did you use adjuvant-pretreated animals as your controls? If not, do you think there is a possibility that some of the reactions may be photoirritation rather than photoallergy?

L. C. Harber: Our findings in patch test studies with humans regarding the "angry back" syndrome or "nonspecific" positive patch tests are in accord with those described by Dr. Mitchell. In terms of guinea pigs, however, we have not observed this phenomenon. Unfortunately, I have no direct experience with the Magnusson test, but our photobiology group has assessed in guinea pigs the angry back syndrome in regard to two photosensitizing fragrance materials, musk ambrette and 6-methylcoumarin. Specifically, relevant control studies concerning induction of photosensitization with the use of the Freund's adjuvant technique presented here involved an innocuous agent, cupric sulfate ($CuSO_4$). This compound has no known primary irritant or allergic properties in humans and thus was chosen for our control studies. Two weeks after attempted induction with $CuSO_4$, the animals were challenged simultaneously with 10% $CuSO_4$, 10% musk ambrette, and 10% 6-methylcoumarin. Each animal was assessed in the presence and absence of UVA light; 6 test sites were evaluated in each of 6 guinea pigs. No reactions were seen at any of the 36 sites.