0022-202X/81/7606-0498$02.00/0
THE JOURNAL OF INVESTIGATIVE DERMATOLOGY, 76:498-501, 1981

Vol. 76, No. 6
Printed in U.S.A.

Photoallergic Contact Dermatitis in Guinea Pigs: Improved Induction Technique Using Freund's Complete Adjuvant

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Efforts to predict the incidence of photoallergic contact dermatitis in man have been hampered by limitations in the animal which have been developed to date. This study reports an improved induction technique in guinea pigs which correlates well with observed clinical experience in man.

New chemicals, including a variety of cosmetic, household, and industrial products, have been introduced to our environment to improve the quality of life. Unfortunately, adverse effects are occasionally noted; photoallergic contact dermatitis is one of these undesired effects. While the incidence of photoallergic contact dermatitis appears to be low, [1, 2] additional cases continue to be found, [3–5] reflecting the increasing diagnostic awareness, and probably also the increasing prevalence, of this phenomenon.

One reason why morbity from photoallergic contact dermatitis continues to occur in the general population is that most of our knowledge of the photoallergic potential of chemicals is obtained from retrospective studies. In addition, there is no adequately reliable animal model for predicting or identifying all the chemicals with photoimmunologic potential for man. Limitations of existing animal models also hamper efforts to better understand the pathogenesis of this problem. To illustrate, 2 widely used fragrance compounds, musk ambrette and 6-methyl coumarin, have recently been recognized as photoallergens. [3, 4] The structure of these compounds is shown in Figure 1. Neither of these compounds photoimmunized guinea pigs using a previously established and widely used procedure for inducing photoallergic contact dermatitis to tetracholosalicylanilide (TCSA) and other halogenated salisylanilides.[6] It is noteworthy that this previously developed techniques had been reproduced by several different laboratories [7–10]. However, it proved necessary to modify this guinea pig model by adding cellophane tape stripping (maximization procedure) as a prerequisite for the induction of photoallergic contact dermatitis to musk ambrette. Even with this modification, the model was unsuccessful in demonstrating photoallergic contact dermatitis to 6-methyl coumarin. [13]

Because of the need for a more reliable model, techniques were sought to develop an animal system that would better predict the photoallergic potential of environmental agents to which man is exposed. This paper reports progress in developing a more reliable guinea pig model for predicting photoallergic contact dermatitis in man.

MATERIALS AND METHODS

Chemicals

Two compounds, musk ambrette and 6-methyl coumarin, were tested. Musk ambrette, 2-methoxy-3,5-dinitro-4-methyl-t-butylbenzene, (Lot #2885-78, Givaudan Corp., Clifton, N.J.) was assayed for purity using high pressure liquid chromatography with detection at 260, 300, or 360 nm. Data indicated that it was at least 99.8% musk ambrette. The 6-methyl coumarin (provided by the Research Institute for Fragrance Materials) was found to be of similar purity. Reagent grade acetone was used as the vehicle for musk ambrette and reagent grade ethanol or ethanol-acetone (1:1) was used as the vehicle for 6-methyl coumarin.

Ultraviolet Radiation sources

Ultraviolet radiation sources were banks of 4 fluorescent tubes, either Westinghouse FS-40 "Sunlight" tubes emitting predominantly in the 285 to 320 nm range (UVB) or General Electric "Black Light" tubes emitting in the 320 to 400 nm range (UVA). The spectral emission of such fluorescent tubes is shown in Fig 2. The output of these sources was quantified with an International Light IL600A Research Photometer using a PT-10C detector with WB-320 and QND-3 filters. The half-power points of this combination of detector and filters are 257 and 392 nm; the instrument was calibrated at 350 nm to a source traceable to the National Bureau of Standards.

Experiment Animals

Hartley strain albino guinea pigs weighing between 350 and 450 gm were used.

Studies for Primary Irritant and Phototoxic Reactions

Musk ambrette was applied in duplicate to the shaved and depilated lumbar area of 10 normal guinea pigs in concentrations of 10, 1.0, and 0.1%. One site was covered with opaque material to test for primary irritant reaction; the other site was irradiated with 10.2 J/cm^2 of UVA to test for phototoxicity. Reactions were scored at 24, 48, and 72 hr according to the following scheme: equivocal or no reaction (0); mild, uniform erythema without edema (1+); erythema with definite edema (2+); or erythema with marked edema (3+). Identical studies were done with 10 other guinea pigs using 6-methyl coumarin.

Studies for Inducing Photoallergic Contact Dermatitis: Method 1

The nuchal area was shaved and depilated (Neet, Whitehall Laboratories, New York, N.Y.). Cellophane tape was used to strip the nuchal epidermis until it glistened. The test material, either musk ambrette or 6-methyl coumarin was applied to the site, which was then irradiated with 6.6 J/cm^2 of UVB and 10.2 J/cm^2 of UVA. The process was repeated for a total of 5 times within 2 weeks, and is summarized in Table I.

Studies for Inducing Photoallergic Contact Dermatitis: Method 2

The nuchal area was shave and depilated (Neet, Whitehall Laboratories, New York, N.Y.). An area of 6-8 sq cm was defined by 4 injections of Freund's complete adjuvant (0.1 ml) into the "corners" (Fig 3). The test material, either musk ambrette or 6-methyl coumarin, was then applied to the area within the "perimeter" defined by the injection sites. Next the area was exposed to 10.2 J/cm^2 of UVA radiation. The adjuvant was only injected once at the time of the initial induction exposure, but the remaining procedures were repeated for a total of 5 times within a 2-week period (Table I).

Procedure for Eliciting Photoallergy

Two weeks after the completion of the induction procedure, guinea pigs were prepared for challenge with the inducing chemical. The lumbar area, which had not been previously treated, was shaved and depilated (Neet, Whitehall Laboratories, New York, N.Y.). The test material was applied in duplicate in concentrations as indicated in Table II. One side received 10.2 J/cm^2 while the other was masked with opaque material as schematically illustrated in Fig 4. Each animal was

Manuscript received August 20, 1980; accepted for publication December 17, 1980.

This work was supported by Grant No. ES01041-07 from the National Institutes of Environmental Health Sciences; Grant No. RR00545 from the National Institutes of Health; and the Shiseido Company, Ltd, Tokyo, Japan.

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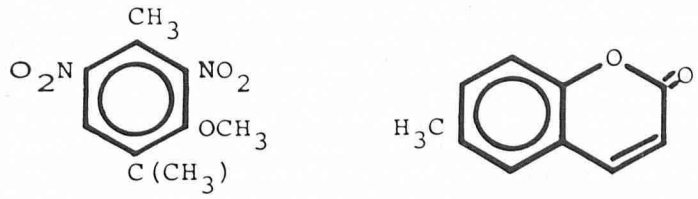


MUSK AMBRETTE 6-METHYL COUMARIN

FIG 1. Molecular structures of musk ambrette and 6-methyl coumarin.

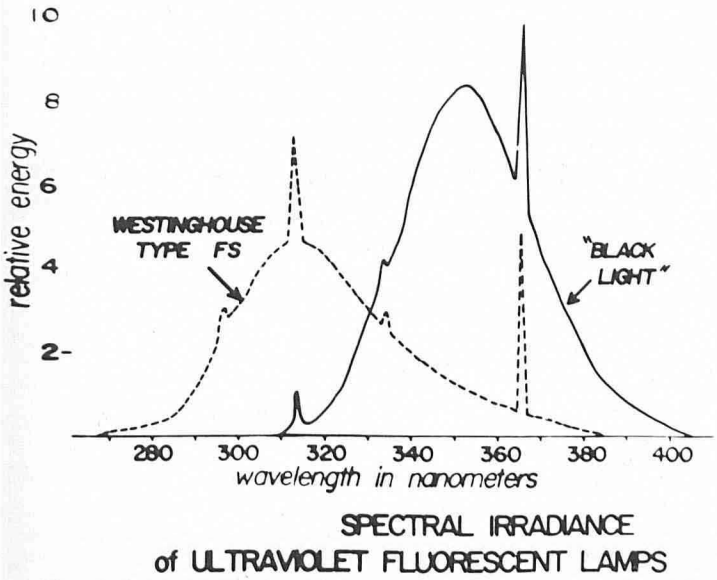


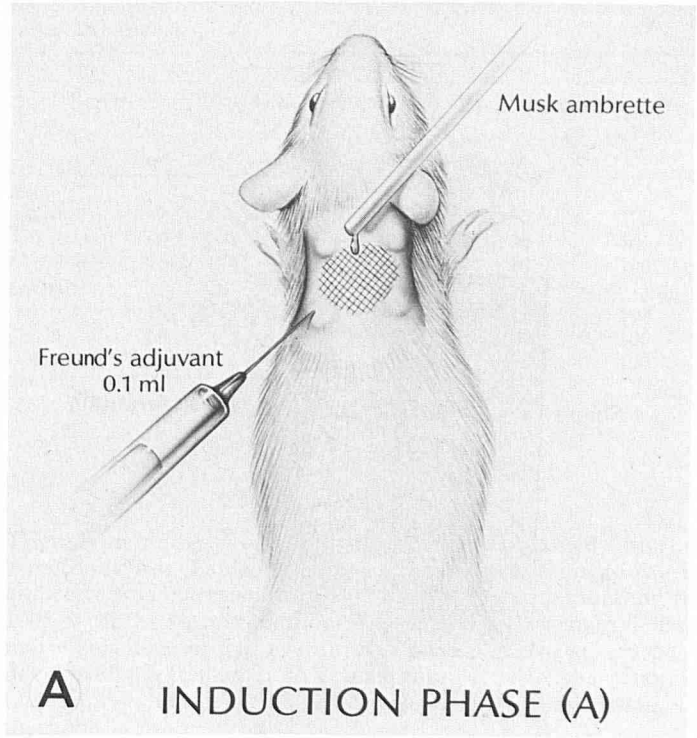
FIG 2. Spectral emission of fluorescent tube sources of ultraviolet radiation.

TABLE I. *Procedure for induction and elicitation of photoallergic contact dermatitis to musk ambrette and 6-methyl coumarin*

Stage	Method 1	Method 2
Induction	1. Shave and depilate.	1. Shave and depilate.
	2. Cellophane tape stripping.	2. FCA 0.1 ml in 4 sites.
	3. Apply test material.	3. Apply test material.
	4. UVB: 6.6 J/cm^2.	4. Remove test material with acetone.
	5. UVA: 10.2 J/cm^2.	5. UVA: 10.2 J/cm^2.
	Repeat steps 1 through 5 for a total of 5 times.	Repeat steps 1, 3, 4, and 5 for a total of 5 times.
Elicitation	1. Shave and depilate.	1. Shave and depilate.
	2. Apply test material for other.	2. Apply test material for other.
	3. Irradiate with UVA (10.2 J/cm^2).	3. Irradiate with UVA (10.2 J./cm^2).

UVA = ultraviolet radiation (320–400); UVB = ultraviolet radiation (290–320); FCA = Freund's complete adjuvant.

assessed for reaction at 24 and 48 hr. Test sites were scored from 0 to 3+ indicating equivocal or no reaction (0); mild, uniform erythema without edema (1+); erythema with definite edema (2+); or erythema with marked edema (3+). The identical elicitation procedure was used for both compounds and was tested two weeks after completion of the respective induction methods (Table I).



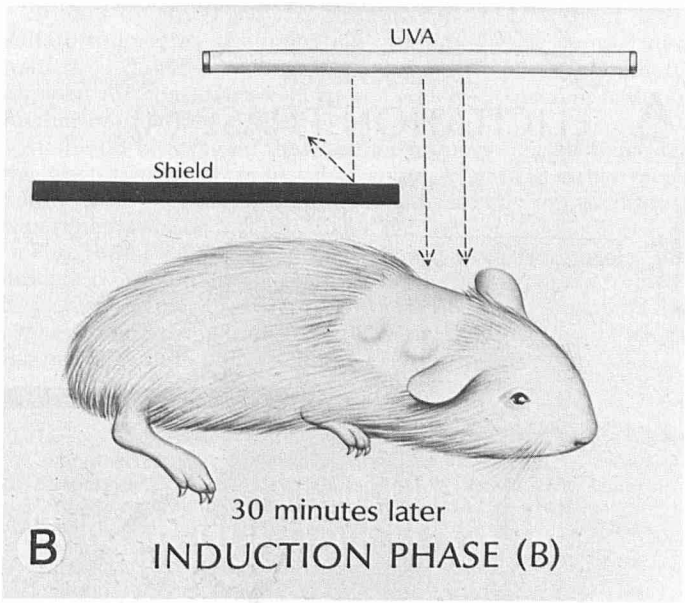


FIG 3. *A*, Schema for inducing photoallergic contact dermatitis using Freund's complete adjuvant. Freund's complete adjuvant is injected into the corners of the induction site and musk ambrette applied to the *cross-hatched* area. See text for details. *B*, Schema for inducing photoallergic contact dermatitis using Freund's complete adjuvant. Induction site is irradiated with 10.2 J/cm^2 of UVA. Note that area of back which will be used for the elicitation phase is shielded.

TABLE II. *Assay for phototoxic photoallergic contact dermatitis to musk ambrette and 6-methyl coumarin*

Musk ambrette			6-Methyl coumarin		
Conc. (%)	UVR dose (J cm^2)		Conc. (%)	UVR dose (J cm^2)	
	10.2	None		10.2	None
10.0	0/10[a]	0/10[a]	10.0	0/10[a]	0/10[a]
1.0	0/10	0/10	1.0	0/10	0/10
0.1	0/10	0/10	0.1	0/10	0/10

[a] = Results given as: Number of animals with erythematous or edematous reaction/number tested.

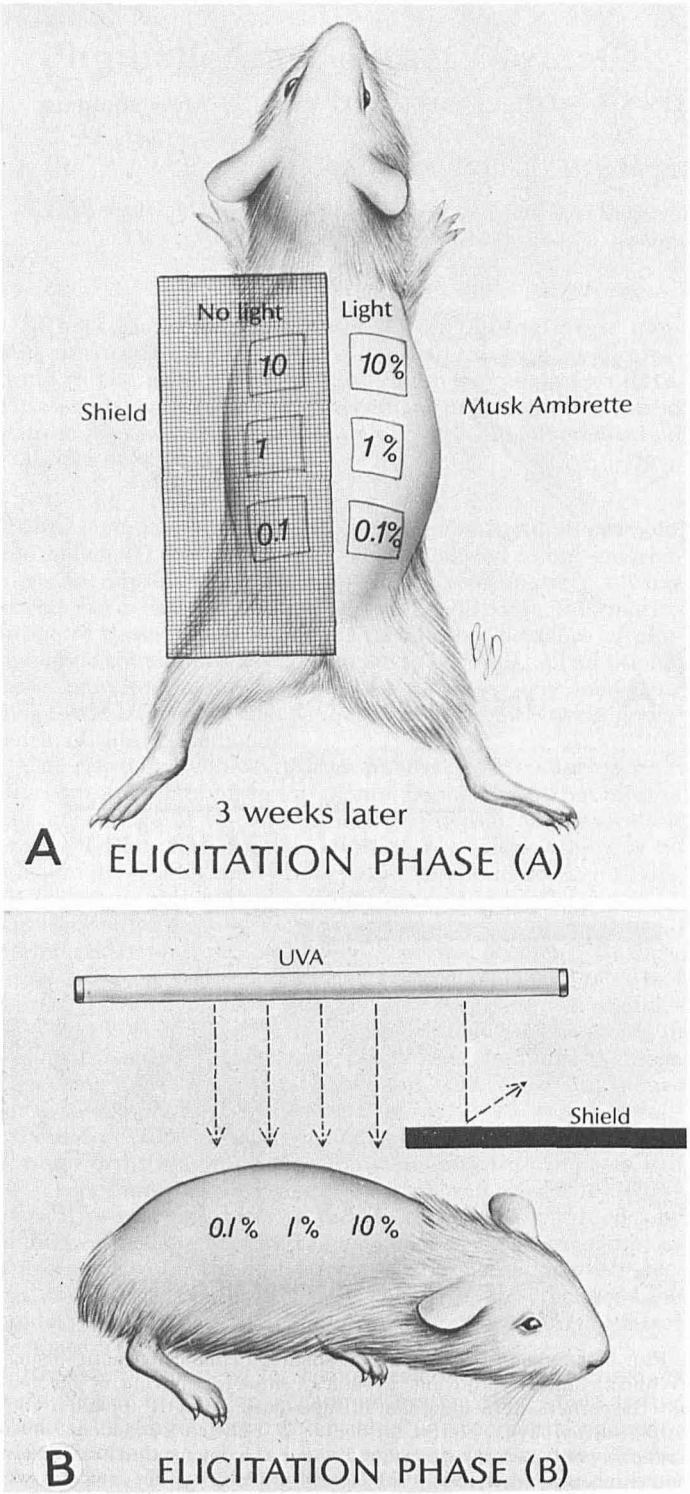


FIG 4. *A*, Elicitation phase. Musk ambrette is applied in duplicate at concentrations of 10%, 1%, and 0.1%. Opaque shielding is used to cover half of the sites as a control for contact dermatitis. *B*, Elicitation Phase. The uncovered half of the sites are irradiated with 10.2 J/cm^2 of UVA. Note that the induction site is shielded during this phase of the procedure.

RESULTS

Tests for primary irritancy and those for phototoxicity to musk ambrette and 6-methyl coumarin were assayed for erythema and edema at 24, 48, and 72 hr. None of the experimental animals showed any evidence of erythema or edema. The 24-hr results, noted in Table II, are representative. The 2 different methods of inducing photoallergic contact dermatitis were compared in order to obtain a relative indication of the index of photosensitization for musk ambrette and 6-methyl coumarin. An identical elicitation procedure was used following both induction procedures for the 2 compounds. The results of these experiments are summarized in Table III. No reaction was detected at any of the unirradiated, control sites with either musk ambrette or 6-methyl coumarin irrespective of the induction procedure. Thus, there was no evidence of primary irritant, phototoxic, or allergic contact dermatitis in guinea pigs to either of these compounds.

The *first induction method*, the one previously reported with musk ambrette, [6] photosensitized approximately 30% of the guinea pigs tested. Photoallergic contact dermatitis to 6-methyl coumarin, however, could not be demonstrated in any of the test animals using this identical method.

The *second method* of inducing photoallergic contact dermatitis to musk ambrette in the guinea pigs tested. Moreover, positive reactions could be detected in all animals with the concentration of musk ambrette as low as 0.1%, as shown in Fig 5. This method was then evaluated for its ability to induce photoallergic contact dermatitis to 6-methyl coumarin. More than 85% of animals tested developed photoallergic contact dermatitis. Moreover, the sensitivity could still be detected in 45% of sensitized animals using 1.0% 6-methyl coumarin, although no reaction was seen using the 0.1% concentration.

DISCUSSION

There is increasing awareness of the photochemical interactions between ultraviolet radiation and chemicals to which man is exposed daily. For example, contact photosensitivity has recently been reported to 6-methyl coumarin, as found in a topical sun protective cream, and to musk ambrette, as incorporated into after shave lotions [11,12]. Similar photoallergic contact dermatitis to 6-methyl coumarin has been induced experimentally in man by Kligman using the "maximization test." However, this identical procedure has been unsuccessful in inducing contact photosensitivity to musk ambrette (A. Kligman, personal communication).

When available, an animal model is a desirable alternative to human investigation and certainly should precede a "usage test." Ideally, an animal model designed to assess this type of reaction would accurately reflect the increasing incidence of contact photoallergic dermatitis. Such a model should not be overly sensitive, indicating irrelevant false positives, but neither should it fail to detect potential hazzards for man with false negatives. Although our studies are preliminary, and a number of additional compounds must be tested, the use of Freund's complete adjuvant shows promise of fulfilling the attributes desired for a predictive model for photoallergic contact dermatitis in man.

In historical perspective, early studies by Schwartz and Speck suggested that guinea pigs were suitable for an animal model to investigate contact photosensitivity to sulfonamides [13]. In 1966, Vinson furthered this animal model by inducing photoallergic contact dermatitis to halogenated salicylanilides [10]. With subsequent modifications this model has been successfully reproduced by several laboratories, including our own [7–10]. Recent attempts employ this technique to induce photoallergic contact dermatitis to musk ambrette, however, were unsuccessful. Cellophane tape stripping at the induction site was necessary in order to reproduce photoallergic contact dermatitis to musk ambrette [6]. In contrast, this same technique could not be successfully employed for inducing photoallergy to 6-methyl coumarin. Thus no procedure has as yet been developed which is a consistently reliable predictor in man or animals of a given compound's photoimmunologic potential. Rather, each technique offers useful features in a given context.

Because preparation of the induction site with cellophane tape stripping and UVB irradiation proved of limited effectiveness, this technique (method 1) was modified by an initial intradermal injection of Freund's complete adjuvant and omission of cellophane tape stripping and the UVB (method 2).

TABLE III. *Comparison of photosensitization to musk ambrette and 6-methyl coumarin by 2 induction methods (at least 10 animals tested per group and erythema observed at 48 hr)*

Method 1						Method 2					
Conc. (%)	% with reaction after UVA[a] (10.2 J/cm²)[b]				No UVA nr	Conc. (%)	% with reaction after UVA[a] (10.2 J/cm²)[b]				No UVA nr
	nr[c]	1+[d]	2+	3+			nr[c]	1+[d]	2+	3+	
					Musk Ambrette						
7.0	70	20	10	0	100	10.0	0	0	20	80	100
5.0	80	20	0	0	100	1.0	0	10	30	60	100
						0.1	0	60	30	10	100
					6-Methyl coumarin						
10.0	100	0	0	0	100	10.0	15	85	0	0	100
						1.0	55	45	0	0	100
						0.1	100	0	0	0	100

[a] UVA = ultraviolet light (320–400 nm).
[b] J/cm² = Joules per square centimeter.
[c] nr = no reaction.
[d] See Methods section for key to criteria for grading reaction 1+ to 3+.

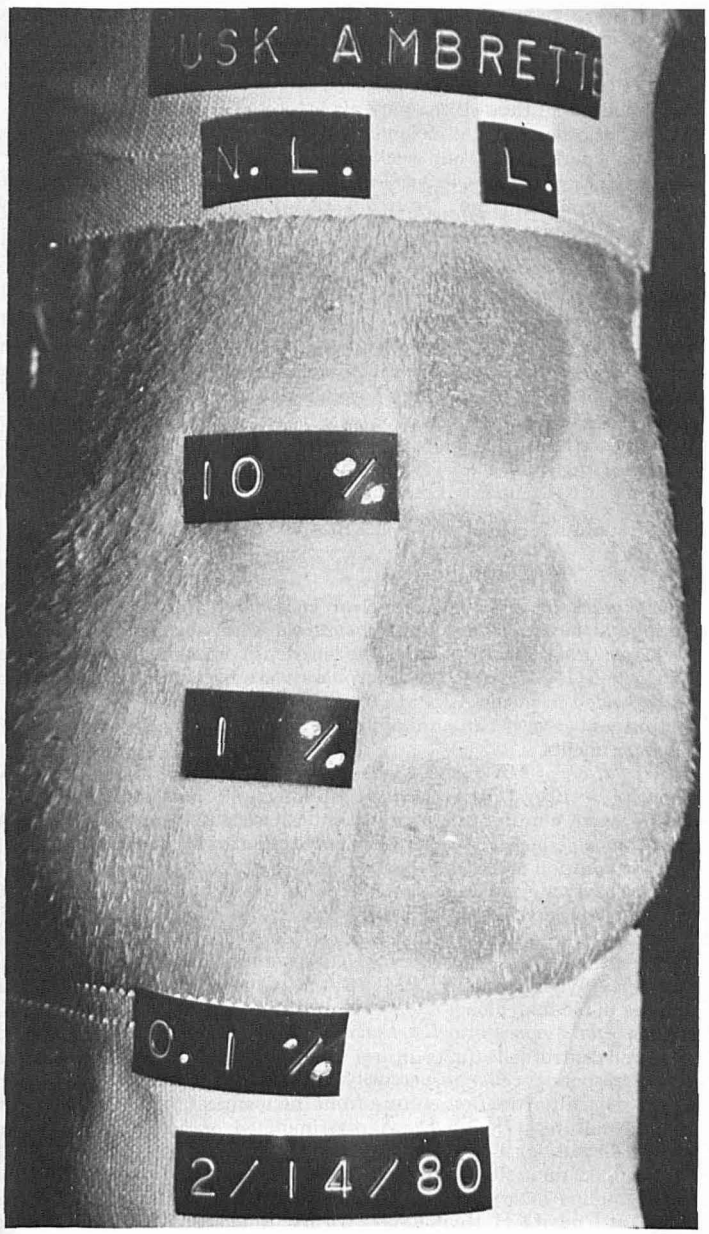


FIG 5. Results of photopatch testing photoallergic contact dermatitis. This guinea pig presents representative results of the new procedure described. Positive reactions occurred only in the sites on the right, the sites which were exposed to ultraviolet radiation.

There is an impressive contrast in the results obtained with the 2 methods (see Table III). The rate of sensitization to musk ambrette was increased from 30% with the original method to 100% with the second method. The results with 6-methyl coumarin sensitization are even more impressive. No guinea pigs developed photoallergic contact dermatitis with the original technique, while over 85% were sensitized with the modified method.

Studies by Horio indicate promise for use of sodium laurel sulfate to enhance the induction of photoallergic contact dermatitis [14]. Further investigation of this agent should be pursued for comparative purposes and to determine if it can further improve the model.

It should be stressed that neither primary irritant reactions nor phototoxicity were found in guinea pigs with either musk ambrette or 6-methyl coumarin, as indicated by representative experiments summarized in Table II.

The ability of Freund's complete adjuvant to enhance the induction of photoallergic contact dermatitis promises to facilitate further investigation into its mechanism. It should be expanded and compared with additional modifications, such as the use of sodium laurel sulfate.

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