the possibilities and ramifications are manifest. The approach of incubating the drug with intact tissue before homogenization and assay may be a useful one and should be considered even when quantitative extraction from a homogenate is obtained.

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*Keyphrases*

Drug estimation—biologic tissue
Intestinal homogenates—phenol red recovery
Intestinal sacs, intact—phenol red recovery
Colorimetric analysis—spectrophotometry

# Method for Testing the Efficacy of Topical Sunscreen Preparations

By W. M. KOOYERS

**A method has been developed for testing the efficacy of topical sunscreen preparations using photosensitive albino rats. The method involves pretreatment of one hind paw with a sunscreen preparation, followed by an oral dose of a photosensitizing agent and exposure to direct sunlight. The delayed reaction and resulting difference between the treated and untreated hind paw weight is an objective index of the protection afforded by the preparation tested.**

INTEREST IN topical sunscreen preparations was stimulated by the successful treatment of light-sensitive patients at the University of Minnesota Hospital. Fusaro and Runge (1–3) reported clinical data supporting the hypothesis that topical treatment of the stratum corneum can reduce or minimize the harmful effect of ultraviolet radiation.

The researcher's never-ending quest for new or better means to treat human diseases has led to the development of biological systems which simulate these diseases. As the diabetic rat is used as a screen for antidiabetic drugs (4), and the adjuvant arthritic rat for testing anti-inflammatory drugs (5), this method uses a photosensitive rat to test the efficacy of topical sunscreen preparations. A sunscreen preparation (SSP) is defined as a formulation which, when applied topically, protects the treated area from sunburn.

Albino rats are not hyperphotosensitive; however, rats treated orally with 25 mg./Kg. of 8-methoxypsoralen (8-MOP) become very photosensitive (6–9). Subsequent exposure of 3–7 hr. of direct sunlight will initiate a delayed reaction that progressively worsens. The resulting erythema, edema, and blindness will incapacitate these rats after 5–7 days. Rats photosensitized with 8-MOP, which have only part of their extremities exposed to direct sunlight, will show evidence of photosensitivity on the exposed areas only (9).

This photosensitive reaction is evident 2 or 3 days after exposure and can be visually evaluated by scoring the affected extremities from 0 to 4 depending upon the severity of the erythema and edema. A more objective index is the comparison of hind paw weights of photosensitive rats and normal rats. This is a measurement of the edema caused by the reaction. This objective index is used in this method.

## METHOD

Five male albino rats[1] per group, weighing 180–200 Gm., were restrained in stocks while each left hind paw was dipped into the SSP. The restraint was maintained until the treated area was completely dry. This minimized the possibility of systemic effect that could be caused by ingestion if the rats were allowed to clean the treated area.

After application of the SSP, the sunscreen groups and one control group were dosed orally with the photosensitizing agent, 8-MOP[2] at 25 mg./Kg., and were then placed in direct sunlight for 5 hr. Each group was housed in a cage designed to allow maximum exposure to the sunlight. Normal controls were exposed simultaneously.

Three to seven days after exposure, the rats were sacrificed, body weights recorded, and both hind paws were uniformly severed (10) using a suitable apparatus.[3] The weight of each hind paw was recorded. The weight difference between the untreated right hind paw and the treated left hind

Received February 26, 1968 from the Upjohn Company, Kalamazoo, MI 49001
Accepted for publication March 13, 1968.
The assistance of Dr. N. W. Dunham with this manuscript and the technical assistance of D. Stapert and W. L. Bogue are acknowledged with appreciation. The experience and background gained by the author while working with Dr. E. M. Glenn of the Pharmacology Department of The Upjohn Company contributed greatly to this work.

[1] Spartan Research, Sprague-Dawley strain.
[2] Five mg./ml. of 8-MOP as a suspension containing per ml.: 5 mg. sodium carboxymethylcellulose; 4 mg. polysorbate 80; 9 mg. sodium chloride; and 9 mg. benzyl alcohol NF.
[3] Harvard Apparatus Company's decapitator.

TABLE I—EFFECT OF SUNSCREEN PREPARATIONS ON HIND PAW WEIGHTS OF PHOTOSENSITIVE RATS

| Experiment | Treatment: Day 1 Group No. and Title | Day 2: 8-MOP[b] | Day 2: 5 hr. Sunlight | Av. Wt., in Gm.: 5 Rats/Group: Final Body Weights ± SEM[c] | Right Hind Paw ± SEM | Left Hind Paw ± SEM | Difference ± SEM |
|---|---|---|---|---|---|---|---|
| No. 1 | 1 Normal controls | No | Yes | 206 ± 2 | 1.39 ± 0.02 | 1.40 ± 0.02 | −0.01 ± 0.01 |
| | 2 Photosensitive controls | Yes | Yes | 180 ± 2 | 2.08 ± 0.03 | 2.06 ± 0.04 | 0.02 ± 0.05 |
| | 3 Sunscreen A[a] | Yes | Yes | 181 ± 1 | 1.91 ± 0.07 | 1.35 ± 0.04 | 0.56 ± 0.05 |
| | 4 Sunscreen B[a] | Yes | Yes | 180 ± 1 | 1.92 ± 0.07 | 1.34 ± 0.02 | 0.58 ± 0.07 |
| No. 2 | 5 Normal controls | No | Yes | 224 ± 1 | 1.43 ± 0.07 | 1.45 ± 0.02 | −0.02 ± 0.01 |
| | 6 Photosensitive controls | Yes | Yes | 185 ± 6 | 1.80 ± 0.06 | 1.81 ± 0.08 | −0.01 ± 0.02 |
| | 7 Sunscreen C[a] | Yes | Yes | 178 ± 2 | 1.93 ± 0.07 | 1.48 ± 0.05 | 0.45 ± 0.02 |
| | 8 Sunscreen D[a] | Yes | Yes | 174 ± 4 | 1.61 ± 0.07 | 1.33 ± 0.01 | 0.28 ± 0.06 |

[a] Left hind paws dipped into the sunscreen preparation six times on Day 1. [b] 8-Methoxypsoralen 5 mg./ml. suspension given orally at 8:30 a.m. Day 2. [c] SEM = Standard error of the mean. Experiment No. 1 terminated 3 days after exposure. Experiment No. 2 terminated 4 days after exposure.

TABLE II—HIND PAW WEIGHT DIFFERENCE EVALUATED BY THE *t* TEST ON DIFFERENCES

| Group No. and Title | Av. Difference | Sum of Difference | Calculated[a] *t* | Allowed *t*[b]: $p = 0.05$ | Allowed *t*[b]: $p = 0.01$ | Significant |
|---|---|---|---|---|---|---|
| 1 Normal controls | −0.01 | −0.05 | 1.00 | 2.78 | 4.60 | |
| 2 Photosensitive controls | 0.02 | 0.10 | 0.42 | 2.78 | 4.60 | |
| 3 Sunscreen A | 0.56 | 2.82 | 10.84 | 2.78 | 4.60 | $p = 0.01$ |
| 4 Sunscreen B | 0.58 | 2.88 | 9.31 | 2.78 | 4.60 | $p = 0.01$ |
| 5 Normal controls | −0.02 | −0.08 | 1.55 | 2.78 | 4.60 | |
| 6 Photosensitive controls | −0.01 | −0.03 | 0.27 | 2.78 | 4.60 | |
| 7 Sunscreen C | 0.45 | 2.25 | 15.33 | 2.78 | 4.60 | $p = 0.01$ |
| 8 Sunscreen D | 0.28 | 1.42 | 4.85 | 2.78 | 4.60 | $p = 0.01$ |

[a] Calculation performed by Control Statistical Services, the Upjohn Company. [b] Allowed *t* values are extracted from Table III of Fisher and Yates, Statistical Tables, at 4 *df*.

paw was an objective index of the protection afforded by the SSP. The statistical significance of this weight difference was evaluated by the *t* test on differences.

## RESULTS AND DISCUSSIONS

Results presented in Table I are typical for this method. The body weights were routinely recorded to help identify aberrant rats. Normal and photosensitive controls are used to prove the sensitivity of this biological system. The hind paw weights within each control group are uniform; however, the photosensitive controls weigh considerably more than normal controls. This edematous condition of the photosensitive rats occurred 3–7 days after the oral dose of 8-MOP and exposure to direct sunlight.

The efficacy of the SSP is evident in Groups 3, 4, 7, and 8 of Table I. The average weight of sunscreen-treated left hind paws is similar to that of normal controls, while the untreated right hind paws are edematous like the photosensitive controls. The calculated weight difference between the untreated and treated paws of each rat is evidence of the protection afforded the treated paw by the SSP.

Using five rats per group, this weight difference is statistically significant at $p = 0.01$ when evaluated by the *t* test on differences. These statistical data are reported in Table II.


## SUMMARY

A method for testing the efficacy of topical sunscreen preparations is reported. The hind paw weight difference of photosensitive rats is a statistically significant index, easily controlled, and objective.


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*Keyphrases*

Sunscreen preparations—testing
8-Methoxypsoralen—photosensitizer
Protection index—rat paw weights