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THE EFFECT OF HUMIDITY ON THE PHOTOTOXIC RESPONSE TO 8-METHOXYPSORALEN IN GUINEA PIGS

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Temperature and relative humidity are most important variables in mediating skin reactions produced by topically applied substances. Renshaw in 1947 (5) demonstrated that both heat and humidity intensified the vesicating action of mustard gas on human skin. Fritsch and Stoughton (3) have reported that the penetration of C^{14} acetylsalicylic acid is enhanced by high temperature and high relative humidity. More recently, Harber and Baer (4) have studied the role of humidity in photosensitivity reactions. They showed that at constant temperatures, variations of the relative humidity had profound influence on the intensity of the phototoxic response to 8-methoxypsoralen (8-MOP).

The present study was carried out to further quantitate the effect of variations of relative humidity on the photosensitive reaction produced by 8-MOP administered topically when other factors such as light intensity, concentration of 8-MOP and temperature were kept constant.

Materials and Method

Experimental Animals: 54 Hartley strain albino guinea pigs weighing 325 ± 25 grams were used. Each animal was housed in a separate cage.

Temperature and Humidity: All animals receiving topical application of 8-MOP and irradiation were exposed in a controlled temperature-humidity room. The animals were brought into the room in pairs; 8-MOP then was applied and the animals irradiated 30 minutes after entry into the room. Temperature in the temperature-humidity room was maintained at $35 \pm 2°C$. Relative humidity was varied from 30 ± 2 % to 85 ± 2 %.[1] Following exposure to experimental conditions, the guinea pigs were returned to the animal room where the temperature was 28°C and the humidity between 40 % and 60 %.

Topical Application: Animals were depilated with a barium sulfide-zinc oxide-cornstarch mixture and their backs divided into quadrants. 0.5 ml of the appropriate 8-MOP solution was applied to each quadrant. Six concentrations of 8-MOP were used: 3×10^{-3} M/l; 1.5×10^{-3} M/l; 7.5×10^{-4} M/l; 3×10^{-4} M/l; 1.5×10^{-4} M/l; 3×10^{-5} M/l. The vehicle was 95 % ethanol. These concentrations were found to be non-primary irritants for the skin of non-irradiated control animals.

[1] Pilot studies indicated that guinea pigs exposed to a relative humidity above 85 % incurred a high mortality rate.

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Table 1. *The phototoxic response of 8-methoxypsoralen*

Concentration of 8-MOP (M)	Relative humidity (%)													
	30		40		50		60		70		75		85	
3×10^{-3}	2	2	2	2	2	2	2	2	2	2	2	2	2.5	2.5
	2	2	2	2	2	1.5	2	2	2	2	2	2	2	2
													2	2
	(2)		(2)		(2)		(2)		(2)		(2)		(2)	
1.5×10^{-3}	1.5	1	1.5	1	2	2	2	2	2	2	2	2	2	2
	1	1	1	.5	2	2	2	2	2	2	2	2	2	2
					1.5	1.5	2	2	2	2	2	2	2	1.5
	(1)		(1)		(2)		(2)		(2)		(2)		(2)	
7.5×10^{-4}	.5	0	.5	0	1.5	1	2	1.5	2	1.5	2	2	2	2
	0	0	0	0	1	1	1	1	1.5	1	2	1.5	2	2
	0	0	0	0	1	.5	1	1	1	1	1.5	1.5	1.5	1.5
	(0)		(0)		(1)		(1)		(1.5)		(2)		(2)	
3×10^{-4}	0	0	0	0	.5	0	1	.5			1	.5	1	1
	0	0	0	0	0	0	.5	.5	1	.5	.5	.5	1	.5
					0	0	.5	.5	0	0	0	0	.5	.5
	(0)		(0)		(0)		(.5)		(.5)		(.5)		(1)	
1.5×10^{-4}	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3×10^{-5}	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Controls	.5	.5							.5	.5				

Systemic Administration: Four guinea pigs received 5 mg of 8-MOP orally and were irradiated two hours later.

Light Source: The light source was identical to that previously described (4). In essence, the animals received 30 mins. of irradiation from "Black Light" lamps with a peak emission in the 3600 A range, energy output of 1200 microwatts/cm^2, target distance of 25 cm. All exposures were filtered with 3 mm of window glass to eliminate any erythrogenic radiation.

Assessment of Reactions: All animals were observed 24 hours after irradiation. Erythema was judged on an arbitrary scale described previously (4):

0—no erythema
0.5—questionable erythema
1—minimal but definite erythema
2—moderate erythema
3—considerable erythema
4—erythema with edema

Results

Four control guinea pigs given 5 mg. of 8-MOP systemically and irradiated two hours later showed no difference in erythema response at 30 % or 70 % relative humidity (see Table 1).

Erythema Threshold:[2] Table 1 shows the response of fifty guinea pigs exposed to various concentrations of 8-MOP at one of seven different relative humidities. The data in Table 1 indicate that a striking augmentation of the phototoxic response is produced by alterations in both concentration of 8-MOP and relative humidity. Each

[2] The erythema threshold in these experiments was defined as the concentration of 8-MOP required to produce minimal but definite erythema.

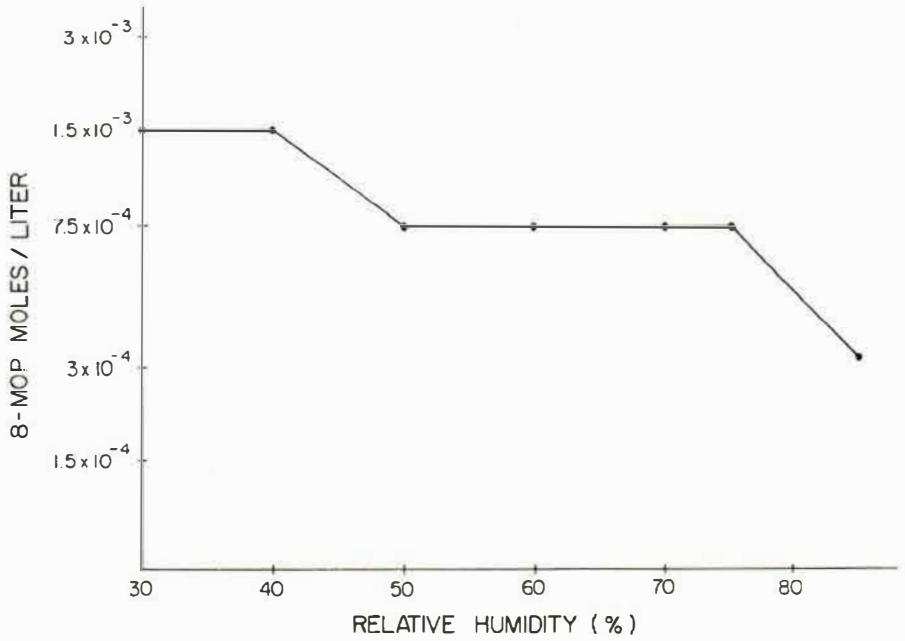

Fig. 1. Concentration of 8-MOP required to elicit threshold erythema (phototoxic responses) at various relative humidities.

set of readings at a particular relative humidity and concentration of 8-MOP was assigned a weighted value (see Table 1).[a] Our studies show that the concentration of 8-MOP needed to produce threshold erythema decreased as the humidity was increased from 30 % to 85 %. Figure 1 diagrams the relationship of concentration necessary to produce threshold erythema under various conditions of relative humidity. A crucial decrease in the erythema threshold occurred between 40 % to 50 % relative humidity and 75 to 85 % relative humidity.

Intensity of Erythema: Table 1 shows that at constant temperature and concentration of 8-MOP the intensity of the erythema response depends on the relative humidity. Table 1 also shows that at any one relative humidity the intensity of the erythema depends on the concentration of 8-MOP. A concentration of 8-MOP of 7.5×10^{-4} M/l was the most sensitive indicator of the phototoxic response and best showed the effect of relative humidity on the intensity of the erythema. High concentrations (3×10^{-3} and 1.5×10^{-3} M/l) produced strong responses throughout the humidity range. Figure 2 illustrates the range of erythema response in the animals tested with the various concentrations of 8-MOP as a function of humidity.

Discussion

This study was designed to pursue further the influence of humidity on phototoxic skin reactions. The exact process by which humidity increases percutaneous absorption has not been elucidated, although recently Scheuplein (6) proposed that percutaneous absorption occurs in two phases; an early short diffusion through follicles and ducts

[a] Weighted values were arbitrarily determined to be:
0—if less than half the responses were less than .5
.5—if less than half the responses were less than 1
1—if less than half the responses were 1.5 or less
2—if at least half the responses were 2 or more

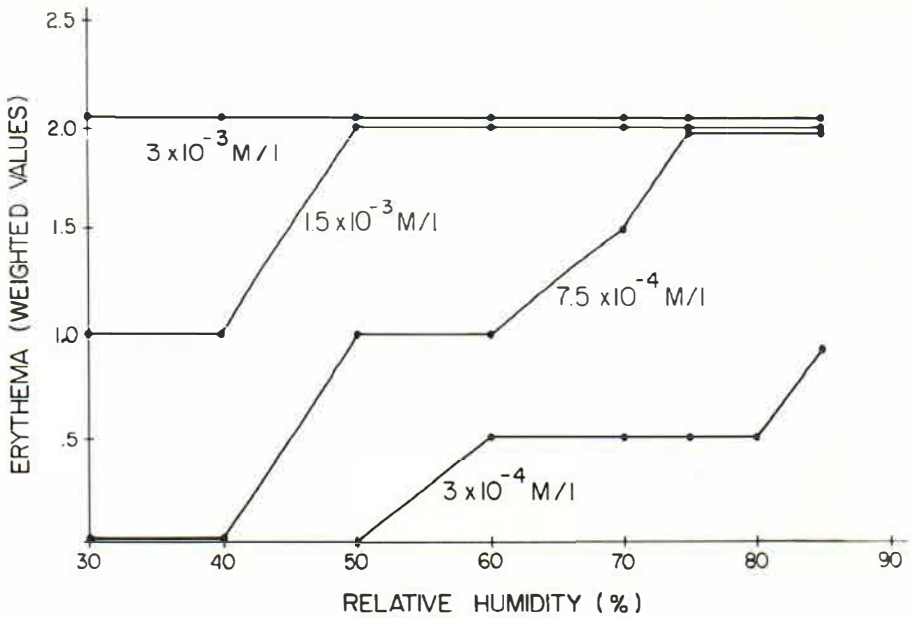

Fig. 2. Influence of relative humidity on the intensity of the phototoxic response to topical administration of 8-MOP.

lasting an estimated 300 seconds, followed by a steady state bulk diffusion through the stratum corneum. Previous studies have shown that high relative humidity enhances the phototoxic effect of 8-MOP (4). In vitro studies by Blank (1) and Cronin & Stoughton (2) have amply demonstrated that small molecular weight compounds do traverse the epidermis at accelerated rates under high temperature and high humidity conditions. We believe that the data presented here represent an in vivo demonstration of the quantitative aspects of percutaneous absorption.

Our studies show that the erythema threshold of the phototoxic response decreases with increasing humidity. Therefore, we may assume that percutaneous absorption of 8-MOP was markedly augmented when the relative humidity was between 40 % and 50 % as compared to 30 % relative humidity. The ratio of 8-MOP absorbed to the amount applied to the skin increases most rapidly between 40 % and 50 % relative humidity. The increased absorption results in a decrease in the concentration of 8-MOP needed to produce a given response. Thus, between 40 % and 50 % relative humidity there was a two-fold drop in the concentration of 8-MOP needed to produce minimal erythema under the experimental conditions of this study from 1.5×10^{-3} M/l to 7.5×10^{-4} M/l.

The intensity of the phototoxic response produced by any one concentration of 8-MOP is similarly dependent on the relative humidity. At a concentration of 7.5×10^{-4} M/l the intensity of the response was minute at low humidity, increased sharply at 40–50 % and was very strong at high humidity. This data suggests that compounds which may well be innocuous at normal temperature and humidity can have hazardous effects when used under environmental conditions of high humidity and high temperature.

SUMMARY

A marked augmentation of the phototoxic reaction to 8-methoxypsoralen occurred between 40 % and 50 % and 75–85 % relative humidity as compared to 30 % relative humidity. Our data suggest increased percutaneous absorption in this range resulted in a greater concentration of the phototoxic agent reaching sites of initiation of the phototoxic response.

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