

A study of oil of bergamot and its importance as a phototoxic agent

II. Factors which affect the phototoxic reaction induced by bergamot oil and psoralen derivatives

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Using a standardized open photopatch test technique, the phototoxic reactions produced by bergamot oil bergapten (5-methoxypsoralen) the active component of the oil, and xanthotoxin (8-methoxypsoralen) were studied. The reaction was affected by a range of factors such as the vehicle (PMF or ethanol), the concentration of ethanol in the vehicle, the skin site, the interval between application of the psoralen and irradiation, the hydration of the skin, and the degree of natural or sun-induced pigmentation. Repeated photopatch testing at the same skin site produced an increase in sensitivity.

Eye colour, natural susceptibility to suntanning, age, and sex, had no effect on the phototoxic response to psoralens.

Key words: Bergamot oil photosensitivity – phototoxic reaction – psoralen phototoxicity.

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Psoralens produce a phototoxic reaction with pigmentation following application to the skin and subsequent irradiation with long wavelength ultraviolet radiation (UVA). The reaction is dependent on the quantity of psoralen applied and the dose of UVA used. Other factors, such as the nature of the vehicle and its mode of application, the skin site involved, the size of the area affected, the interval between application of psoralen and exposure to UVA, hydration of the skin, constitutive and facultative skin colour, and the effect of repeated applications, may affect the frequency and intensity of the response. A study of these factors was felt to be important because of their possible relevance to the development of phototoxic reactions

of the skin such as Berloque dermatitis and abnormal hyperpigmentation, etc.

Materials and Methods

Bergamot oil (Coty) previously shown to contain 0.31 % (w/w) bergapten (5-methoxypsoralen, 5-MOP) was used (Zaynoun et al. 1977). Concentrations of the oil in ethanol and PMF (Paraffin Molle Flavum) vehicles ranged from 0.06 to 20 %. Pure 5-MOP was obtained from Unilever Research Laboratories and pure 8-methoxypsoralen (8-MOP) purchased from Sigma Ltd. Unless otherwise stated, photopatch tests were carried out on the lower thoracic back skin of normal volunteers. Open photopatch tests were carried out by ap-

plying 1.5 microlitre aliquots of test solution or approximately 1.5 mg of PMF containing the appropriate amount of phototoxic compound, evenly to 1×1 cm squares marked on the skin. The same amount of bergamot oil was applied in each site for the various concentrations used irrespective of the vehicle; for example, with a 1 % bergamot oil concentration both 1.5 microlitres of ethanolic solution and 1.5 mg of PMF contained 0.015 microlitres of bergamot oil. One hour later the test sites were irradiated for 30 min at a distance of 10 cm with UVA (Philips TL20W08, F20T12BLB, "blacklight" fluorescent tube, filtered with window glass to give wavelengths 320–400 nm (peak 355 nm)), the total incident dose being approximately 900 mJ/cm². The test sites were examined for reactions at 24, 48, 72, and 96 h after irradiation. In particular, the minimally effective concentration (MEC) and the percent positive response levels were recorded.

Closed or occluded photopatch tests were carried out by applying 25 microlitre aliquots of ethanolic solution or 25 mg aliquots of PMF containing bergamot oil, to standard 1.3×1.3 cm test patches. These patches were then placed on the skin and occluded with black polythene and occlusive acrylic tape (Blenderm MMM®) for 24 h before "blacklight" irradiation. For a comparison of open and closed photopatch tests, concentrations of 0.125, 0.25, 0.50, 1.0, 2.5, 5, 10, and 15 % bergamot oil in both ethanol and PMF were used.

For further assessment of the effect of variation in the vehicle, the open photopatch test technique was used with 0.125, 0.25, 0.50, 1.0, 2.5, 5 and 10 % bergamot oil in absolute ethanol and in a 50 % aqueous solution of ethanol, applied in parallel rows with the corresponding concentrations in the two vehicles in adjacent positions.

The effect of variation in the size of the skin area tested was studied using the open photopatch test technique with a range of bergamot oil concentrations in absolute ethanol (0.25–10 %). Volumes of 1.5 microlitres were applied to 1×1 cm areas and 4.5 microlitres to 3×1 cm areas (i.e. 1.5 microlitres/cm²) in two parallel rows.

To test the effect of variation in skin site, bergamot oil 0.125–10 % concentrations in absolute ethanol were used. Open photopatch tests were carried out using 1.5 microlitre aliquots, one set being applied to the lower thoracic region of the back and another to the lower lumbar region. The two sets were irradiated with "blacklight" simultaneously.

To assess the significance of the interval of time between the application of the psoralen and the subsequent irradiation, open photopatch tests were carried out with bergamot oil, 0.5, 1, 2.5, 5, 10, 15 and 20 % in ethanol, 1, 5, 10 and 15 and 20 % in PMF, and with pure 8-MOP 0.01, 0.1 and 0.5 % in both ethanol and PMF. Using 0.5, 1, 2.5 and 5 % bergamot oil in ethanol, nine separate sets of photopatch tests were applied, 1.5 microlitres for each test, one set being irradiated with "blacklight" immediately and the others being covered with light excluding black paper for subsequent irradiation at $\frac{1}{2}$, 1, 2, 4, 6, 8, 10 and 12 h afterwards. With bergamot oil concentrations of 1, 5, 10, 15 and 20 % in both ethanol and PMF, seven sets were applied for irradiation at 0, $\frac{1}{2}$, 2, 4, 6, 8 and 12 h. For 8-MOP in both ethanol and PMF, 13 sets were applied for irradiation at 0, $\frac{1}{2}$, 1, 2, 4, 6, 8, 10, 12, 18, 24, 36 and 48 h.

To assess the role of the stratum corneum in any delay in reaction which might occur between the time of application and the irradiation, a small area of skin on the lower thoracic region of the back was stripped with cello tape until glistening. Open photopatch tests, using 2.5 and 5 %

bergamot oil in ethanol, were carried out on the stripped skin and on adjacent normal skin, the sites being irradiated with "blacklight" immediately after the application of 1.5 microlitre aliquots.

The effect of hydration on the skin response to psoralens and UVA was studied using two separate techniques. First, the skin on one side of the back was occluded with polythene for 24 h, the other side serving as a control. Two sets of bergamot oil, 1.5 microlitre aliquots of 0.125, 0.25, 0.5, 1.0, 2.5, 5 and 10 % in ethanol, were applied, one set to the previously occluded skin and the other to the control side. The sites on the occluded side were again covered for 1 h with polythene while the control skin remained uncovered. Both sets were then irradiated with "blacklight" radiation and the responses recorded as before. Secondly, a soap and water wash was applied to one side of the back for 5 min and the photopatch tests carried out as before.

To assess the effect of repeated applications of psoralen and "blacklight" irradiation, open photopatch tests with bergamot oil, pure 5-MOP and pure 8-MOP were used. With bergamot oil, 1.5 microlitre aliquots of concentrations ranging from 0.125 to 15 % in ethanol were applied to carefully marked sites on lower thoracic back skin. After 1 h, "blacklight" irradiation was given as before and the skin was examined over the next 4 days to obtain the MEC value. On the fourth day the procedure was repeated, each side receiving the same concentration of bergamot oil as it did in the initial test, and the MEC value was again recorded. With 5-MOP and 8-MOP, ethanolic solutions with a concentration range of from 0.0001 to 0.05 % were used in the same way, but photopatch tests were carried out twice weekly, the MEC value being obtained for the initial test and after four and seven repeat tests.

Results

The age, sex, skin and eye colour, ability to tan, presence of tan, and MEC values for open and closed photopatch tests with bergamot oil in ethanol for the 63 subjects used in this study are shown in Table 1. Skin colour varied from fair, through light brown and sallow, to dark brown and black. For subjects of North European Caucasoid type (Nos. 1-58) there is no apparent correlation between degree of intrinsic, i.e. genetic, pigmentation and the minimum concentration of bergamot oil required to produce a phototoxic reaction. Subjects 59 and 60 were West Indians of Negroid type, subjects 61 and 62 were Mauritians of Asian Caucasoid type, and subject 63 was a Nigerian. These last five subjects all had dark brown to black intrinsic skin colour and all had high values of MEC. The eye colour in the 63 subjects also varied, with the customary association between blue, green and grey eyes with fair skin and brown eyes with darker shades. However, apart from the findings for subjects 59-63, there is no apparent correlation between eye colour and MEC.

The values of MEC obtained in subjects tested when suntanned appear to be higher than when no suntan is present. Table 2 shows the percentage positive response values obtained from photopatch tests with varying concentrations of bergamot oil in ethanol in subjects 3, 26, 27, 28, 41, 43, 44, and 47-51 who were tanned, compared with those for subjects 1-10 who were untanned. With a tan, the lowest concentration required to produce a reaction is 1 % and even with 5 % bergamot oil only a 50 % positive response level was achieved. With no tan, positive responses were obtained with 0.5 % bergamot oil and all the subjects reacted when 2.5 % was used. Subject 3 was tested without and with a sun-

Table 1. The physical characteristics of the 63 subjects tested and the minimum effective concentrations of bergamot oil required to produce a phototoxic reaction

Case No.	Age (yrs)	Sex	Skin colour	Ability to tan	Eye colour	Suntan on back	Minimal effective concentration of bergamot		
							Open test		Closed test
1	23	F	Fair	0	Brown	0	2.5%,	2.5%,	—
2	64	M	Fair	0	Green	0	1.0%		—
3	42	F	L. brown	++	Brown	0, ++	1.0%,	1.0%, 5.0%	10%
4	28	M	Fair	0	Green	0	1.0%,	1.0%	—
5	33	F	L. brown	++	Brown	0	1.0%,	1.0%	—
6	38	F	Fair	0	Blue	0	2.5%,	2.5%	—
7	22	F	L. brown	++	Blue	0	0.5%,	1.0%	—
8	25	F	Fair	+	Blue	0	2.5%,	2.5%	—
9	26	M	Sallow	++	Green	0	2.5%		—
10	28	M	Sallow	+	Blue	0	1.0%		—
11	62	F	Fair	0	Blue	0		—	1.0%
12	47	F	L. brown	++	Brown	0		—	15%
13	29	M	Fair	++	Brown	0		—	2.5%
14	27	F	Fair	++	Brown	0		—	2.5%
15	30	M	Fair	++	Brown	0	2.5%		1.0%
16	22	F	Fair	0	Grey	0		—	1.0%
17	23	F	Fair	++	Green	0		—	1.0%
18	57	F	Sallow	0	Hazel	0		—	5.0%
19	36	F	Fair	0	Brown	0	1.0%		1.0%
20	21	F	Fair	++	Brown	0	2.5%,	1.0%, 0.5%	—
21	36	F	Fair	0	Green	0	1.0%		—
22	18	F	Fair	+	Blue	0	1.0%,	2.5%, 0.5%	—
23	18	F	L. brown	++	Brown	0	2.5%		—
24	39	F	Sallow	++	Hazel	0	1.0%		—
25	21	F	Fair	++	Hazel	0	1.0%		—
26	22	F	Fair	++	Green	+	2.5%,	2.5%	—
27	21	F	Fair	++	Blue	++	>5.0%		—
28	22	F	Fair	+	Green	+	1.0%		—
29	20	F	Fair	0	Blue	0	1.0%		—
30	26	M	Fair	+	Blue	0		—	—
31	47	M	Sallow	++	Blue	0		—	—
32	22	M	Fair	0	Green	0		—	—
33	23	M	—	—	—	—		—	—
34	46	F	Sallow	++	Blue	0	1.0%		—
35	64	M	Fair	+	Grey	0		—	—
36	23	M	Fair	++	Blue	0	0.25%		—
37	58	F	Fair	0	Hazel	0	>5.0%		—
38	25	M	Fair	++	Green	0	0.25%		—
39	20	F	Fair	+	Green	0	10.0%		—
40	29	F	Fair	++	Brown	0	2.5%		—
41	70	M	Fair	++	Blue	+	2.5%		—
42	22	F	Fair	++	Brown	0	2.5%		—
43	27	F	Fair	+	Blue	+	10.0%		—
44	26	M	Fair	++	Blue	+	5.0%		—
45	19	F	Fair	++	Brown	0	1.0%		—
46	18	F	Sallow	++	Brown	0	1.0%		—
47	22	F	Sallow	++	Blue	++	2.5%		—

Table 1 (Contd.)

Case No.	Age (yrs)	Sex	Skin colour	Ability to tan	Eye colour	Suntan on back	Minimal effective concentration of bergamot	
							Open test	Closed test
48	22	F	Sallow	++	Blue	++	5.0%	-
49	19	F	Fair	+	Blue	++	>5.0%	-
50	23	M	L. brown	++	Grey	++	>5.0%	-
51	29	F	L. brown	++	Brown	++	>5.0%	-
52	23	F	Sallow	++	Green	0	2.5%	-
53	23	F	Fair	+	Hazel	0	0.25%	-
54	27	F	Fair	++	Green	0	1.0%	-
55	28	M	Fair	+	Green	0	0.25%	-
56	30	M	Fair	0	Blue	0	0.5%	-
57	23	F	Fair	++	Blue	0	>1.0%	-
58	29	M	Sallow	0	Brown	0	>5.0%	-
59	21	F	D. brown	++	Brown	0	>15.0%	-
60	22	F	D. brown	++	Brown	0	>15.0%	-
61	20	M	D. brown	++	Brown	0	5.0%	-
62	21	M	D. brown	++	Brown	0	>20.0%	-
63	33	M	Black	++	Black	0	>20.0%	-

N.B. L. brown: light brown D. brown: dark brown

Ability to suntan: 0 = No, + = mild tan, ++ = good tan

Suntan present on the back: 0 = No, + = mild, ++ = moderate or marked

- = denotes information not recorded or test not carried out

Cases 59-63 had not sunbathed during the 5 months prior to testing.

Where more than one minimal effective concentration is recorded in the results from open photopatch tests, the figures represent the results of tests carried out at different times. In subject No. 3, for instance, three separate tests were carried out, 2 before and 1 after suntanning.

induced tan and showed a change in MEC from 1 % to 5 %.

For further assessment of the effects of skin colour, ability to tan, eye colour, age and sex differences in the European caucasoid type skin, and MEC of 2 % or lower

was taken as an arbitrary index of susceptibility to psoralen photosensitivity and the numbers of subjects in each class responding at this level were subjected to the chi square test of significance for any difference observed. Subjects with a suntan at

Table 2. The effect of previous suntan on the response to open photopatch tests with various concentrations of bergamot oil in ethanol. The results (minimal effective concentration) are expressed as percent positive response

Concentration of oil of bergamot		0.125%	0.25%	0.5%	1%	2.5%	5%
Percentage of positive reactions	Suntan Present	0	0	0	8	33	50
	Absent	0	0	10	60	100	100

the time of testing, as well as subject Nos. 59–63, were excluded from this analysis because the degree of pigmentation present in the skin had been shown to result in a considerably higher MEC value. No statistically significant difference was found between the minimal effective concentration required to produce a reaction in a fair-skinned individual and that required to produce a similar reaction in a light brown or sallow skinned person; in that with the former a concentration of 1 % or less was found in 66 % and in the latter 62 %. Differences in ability to tan, eye colour, age and sex did not significantly affect responses to bergamot oil.

When the results are expressed as percent positive responses, there appears to be little difference between absolute ethanol and PMF as a vehicle for bergamot oil in both the open, non-occluded test and the occluded test method as used here. However, to be sure of obtaining 100 % positive responses with the occluded test instead of some 80 or 90 %, a concentration of 15 % bergamot oil is required compared with only 2.5 % in the non-occluded test. With both tests a 50 % positive response level was obtained using 1 % bergamot oil.

Observation of the test sites usually showed no reaction at the first reading, i.e. 24 h after irradiation. However, a moderate increase in positive responses was noted at 48 h with a sharp increase at 72 h. The peak number of positive responses was noted at 96 h. In subject Nos. 11, 37, and 38, mild positive responses were noted for the first time only after a delay of 4 days after irradiation. When stronger concentrations (10 and 15 % bergamot oil) were used, reactions were observed at 24 h with progressively increasing intensity throughout the subsequent 2nd, 3rd and 4th days. In some subjects these higher concentrations produced bullous reactions. In a few subjects a mild response was first seen at

72 h which had cleared by 96 h. To obtain accurate information regarding minimal effects, reading at 72 and 96 h is essential.

Four subjects (Nos. 20, 21, 22 and 23) were used to compare the efficacy of 100 % and 50 % aqueous ethanol as a vehicle for bergamot oil in the open photopatch test technique. Whereas 0.5 % bergamot oil in 100 % ethanol elicited no reactions, in 50 % ethanol it produced positive photopatch test responses in three of the four subjects. To obtain reactions in all the subjects tested, only 1 % bergamot oil in 50 % ethanol was required, while 2.5 % bergamot oil was required with the 100 % ethanol vehicle. Moreover, the positive responses obtained with the 50 % ethanol vehicle appeared to occupy a smaller skin area than those with 100 % ethanol.

The size of the skin area used made no difference to the results of open photopatch testing with different concentrations of bergamot oil, as long as the amount of oil per square cm was the same. For example, with 0.125 to 0.5 % bergamot oil none of the four subjects tested (Nos. 20, 22, 24 and 25) produced a positive response either the 1×1 cm or the 3×1 cm sites. However, each subject reacted to concentrations of 1–10 % irrespective of the size of the skin area tested.

Table 3 shows the results obtained when photopatch tests were carried out at two different parts of the back, i.e. at the lower thoracic (mid back) and at the lower lumbar (lower back) regions. Ten subjects (Nos. 1, 3, 4, 5, 6, 7, 8, 20, 22 and 26) were tested; the results are expressed as the percentage of positive responses obtained at the different parts of the back. A higher percentage of positive responses was obtained using the mid-back, the most marked difference being noted with the 2.5 % concentration of bergamot oil for which there was only 40 % positive response recorded on the lumbar region, while a full 100 %

Table 3. The effect of variation in skin site on the response to a range of concentrations of bergamot oil in ethanol using the open photopatch tests. Results are expressed as percent positive response

Concentration of oil of bergamot		0.125%	0.25%	0.5%	1%	2.5%	5%	10%
Percentage of positive responses	Mid-back	0	0	30	60	100	100	100
	Lower back	0	0	20	40	40	60	80

response level was obtained when the lower thoracic region was used.

To study the effect of varying the time between the application of oil of bergamot to the skin and the subsequent irradiation with UVA, five subjects (Nos. 15, 19, 27, 28 and 29) were tested using 0.5, 1, 2.5, and 5 % concentrations of oil of bergamot in ethanol. The results are shown in Table 4 and expressed as the number of subjects producing a positive response for each concentration of oil of bergamot and for each time interval before irradiation. The maximum degree of response was obtained with an interval of 1–2 h, e.g. with 1 % bergamot oil, a 1-h interval resulted in 2 out of 5 positive reactions, and at the 2-h interval 3 out of 5 positive reactions. With 5 % bergamot oil however both time intervals resulted in 5 out of 5 being positive. Intervals longer or shorter produced decreased levels of response and in no instance was a

positive response obtained if the interval between the application of the bergamot oil to the skin and the subsequent irradiation was 10 h or longer. This was true even for the 5 % bergamot oil concentration.

Table 5 shows the results of studies in four subjects (Nos. 30, 31, 32, and 33) in which bergamot oil (1, 5, 10, 15 and 20 % concentrations) in both ethanol and PMF, was used to determine whether a change in vehicle would affect the results obtained at the various time intervals between the application to the skin and the subsequent irradiation. These results have been expressed as the concentration of oil required to produce a positive response at the time interval concerned.

With the ethanol vehicle no responses were obtained when irradiation immediately followed the application, even with 20 % bergamot oil. With the PMF vehicle on the other hand, positive responses were ob-

Table 4. The effect of the time interval between the application of varying concentrations of oil of bergamot and the subsequent irradiation on five subjects expressed as number producing a positive response

Concentration of oil of bergamot in ethanol	Number of positive responses at different time intervals (hours)								
	0	½	1	2	4	6	8	10	12
½%	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
1%	0/5	1/5	2/5	3/5	2/5	1/5	0/5	0/5	0/5
2.5%	0/5	3/5	4/5	3/5	2/5	1/5	1/5	0/5	0/5
5%	0/5	3/5	5/5	5/5	2/5	2/5	2/5	0/5	0/5
Total	0/20	7/20	11/20	11/20	6/20	4/20	3/20	0/20	0/20

Table 5. The effect of time interval between application and irradiation of photopatch tests using oil of bergamot at different concentrations in (A) ethanol and (B) PMF, results expressed as minimal concentration required to produce a reaction

Concentrations of bergamot oil No. 30 No. 31 No. 32 No. 33	The time interval between the application to the skin and subsequent irradiation (hours)															
	0		1/2		2		4		6		8		12			
	E	P	E	P	E	P	E	P	E	P	E	P	E	P	E	P
No. 30	-	-	15%	1%	-	-	15%	>15%	15%	>15%	>20%	>15%	-	-	-	-
No. 31	>20%	5%	5%	3%	5%	10%	10%	>20%	>20%	>20%	>20%	>20%	-	-	-	-
No. 32	>20%	5%	1%	5%	5%	5%	10%	20%	15%	>20%	15%	>20%	>20%	>20%	>20%	>20%
No. 33	-	-	1%	1%	-	-	15%	>15%	>15%	>15%	>15%	>15%	>15%	>15%	>15%	>15%

E = Ethanol
P = PMF

tained with 5–20 % bergamot oil with this same interval. Another difference in response due to the vehicle is demonstrated in that at the 6-h interval no positive responses could now be obtained with PMF, while when ethanol was used, 2 of the 4 subjects still produced positive responses to 15 % bergamot oil.

Five subjects (Nos. 34, 35, 36, 37, and 38) were used to study the effect of variation in interval between application of pure 8-MOP in both ethanol and PMF and irradiation with "blacklight". The results as shown in Tables 6 (ethanol) and 7 (PMF) are expressed as numbers of positive responses at the various time intervals used for 0.01 and 0.1 and 0.5 % 8-MOP. Some positive responses were obtained with irradiation immediately after application with all concentrations in both vehicles. However, with ethanol the number of positive responses increased with concentration, a feature not observed when PMF was used. Maximum response levels were again observed for the 1- and 2-h intervals and again the persistence of the psoralen in the skin when applied in ethanol was greater than when applied in PMF. The total duration of effectiveness of 0.01 % 8-MOP in ethanol was 6 h, a similar result being obtained with the PMF vehicle. However, with 0.1 % and 0.5 % in ethanol responses were still obtained 12 and 36 h after application respectively, while in PMF these concentrations were effective for up to only 6 and 12 h.

In subject Nos. 17 and 30, when the stratum corneum was removed by stripping prior to photopatch testing in which irradiation immediately followed the application of 2.5 and 5 % bergamot oil in ethanol, erythem and oedema, of greater intensity than that produced by the stripping in the non-irradiated control site, was observed. Photopatch tests on adjacent intact skin produced negative results.

Table 6. The effect of the time interval between application and irradiation of the open photopatch tests using various concentrations of 8-methoxypsoralen in ethanol. Results are expressed as positive responses in five subjects

Time interval (hours)	0	1/2	1	2	4	6	8	10	12	18	24	36	48
Number of positive reactions with each concentration of 8-MOP	0.01%	1/5	4/5	5/5	5/5	2/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5
	0.1%	3/5	5/5	5/5	5/5	5/5	5/5	5/5	3/5	0/5	0/5	0/5	0/5
	0.5%	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	4/5	2/5	0/5
	Total	9/15	14/15	15/15	15/15	12/15	11/15	10/15	9/15	8/15	5/15	4/15	2/15

Table 7. The effect of the time interval between application and irradiation of open photopatch tests using various concentrations of 8-methoxypsoralen in PMF. Results are expressed as positive responses in five subjects

Time interval (Hours)	0	1/2	1	2	4	6	8	10	12	18	24	36	48
Number of positive reactions with each concentration of 8-MOP	0.01%	3/5	3/5	5/5	5/5	4/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5
	0.1%	2/5	5/5	5/5	5/5	5/5	3/5	0/5	0/5	0/5	0/5	0/5	0/5
	0.5%	2/5	—	4/4	4/4	4/4	4/4	4/4	1/4	1/4	0/5	0/5	0/5
	Total	7/15	8/15	14/14	14/14	13/14	8/14	4/14	4/14	1/14	0/15	0/15	0/15

Table 8. The effect of twice-weekly open photopatch tests in the same site on minimal effective concentrations of 5-MOP and 8-MOP in ethanol

		Case No.	Number of applications and irradiations			
			1	4	7	
Minimal concentration of 5-MOP or 8-MOP required to produce a positive reaction	5-MOP	40	{	0.0025%	0.0005%	
		41		0.05%	0.005%	0.001%
	MEC					
	8-MOP	40		0.0025%	0.00025%	
		41		0.025%	0.0025%	0.0005%

Six subjects (Nos. 22, 42, 43, 44, 45 and 46) were used to study the effect of hydration by occlusion on the concentration of bergamot oil required to produce reactions. Without hydration prior to phototesting, 10 % bergamot oil was required to produce reactions in all 6, although 2 produced reactions with 1 %, 3 with 2.5 %, and 5 with 5 %. With prior hydration by occlusion all 6 reacted with 1 % bergamot oil, 4 with 0.5 %, and 1 with as little as 0.125 %.

When the skin was washed with soap and water prior to phototesting (subject Nos. 47 and 48) a decrease in MEC from 2.5 to 1 % and 5 to 2.5 % respectively was obtained.

When two photopatch test series were carried out 4 days apart on the same skin sites in subject Nos. 5 and 39, the MEC for bergamot oil in ethanol showed a significant decrease. In subject No. 5 the value changed from 1 % to 0.5 % and in subject No. 39 from 10 % to 2.5 %. The results obtained in subject Nos. 40 and 41 with twice weekly photopatch tests to the same skin sites with both 5-MOP and 8-MOP are shown in Table 8. These results confirm that repeated applications of psoralen and irradiation with "blacklight" decrease the minimal concentration of the applied chemical required to produce a reaction. Thus in

subject No. 41 the original photopatch test required 0.05 % 5-MOP to produce a reaction while after four tests in the same sites only 0.005 % was required, and after seven tests the concentration required was reduced to 0.001 %.

Discussion

Before starting an extensive series of experiments to investigate the various factors which might affect the psoralen-induced phototoxic reaction, the most suitable standard technique had to be established. The conventional closed photopatch test, involving occlusion of the test material for 24 h followed by exposure to UVA, was therefore compared with the open test in which the material is applied to the skin, left without occlusion for a specified time (in our series 1 h) and then irradiated. Although the results obtained were similar for both the closed and open tests, the latter has a number of advantages. The open test avoids occlusion which not only requires a set time of 24 h but may result in broken contact between the test material and the skin, and may be inconvenient for the test subject. The open photopatch test more nearly simulates the natural occurrence of bergapten-induced phototoxic reactions

such as in Berloque dermatitis and related hyperpigmentary states in which occlusion is unlikely to be a factor. Moreover, when care is taken to avoid loss of material by rubbing after it has been applied, the amount of test substance actually in contact with the skin is known in the open test whereas in the closed test complex diffusion processes from the patch test vehicle and its supporting material to the skin are involved and no definite figure can be obtained. The difficulties in interpretation of the closed photopatch test results are illustrated by the fact that similar percent positive response values were obtained in our comparative tests although there was some 17 times more bergamot oil in the closed photopatch test materials than in the open test for each concentration used.

Using the chamber method for photopatch testing with 0.1 % 8-MOP in petrolatum, Suhonen et al. (1976) found that the size of reaction obtained did indeed vary with the amount of test substance used.

The results of both open and closed tests showed little difference whether 100 % ethanol or PMF was used. However, ethanol is preferred to PMF because it is easier to measure in smaller quantities by means of a micropipette. Moreover, for the study of clinical phototoxic skin reactions presenting as erythema and/or hyperpigmentation, it is preferable to use bergamot oil in ethanol as most perfumes are prepared as alcoholic solutions.

The concentration of alcohol itself will vary in perfumes and our results clearly demonstrate that dilution of the ethanol vehicle increases the percentage of positive responses obtained in man, c.f. species difference (Maibach & Marzulli 1975). The larger area of the reactions obtained with 100 % ethanol as the vehicle would appear to result from spreading of the bergamot oil after application and, as suggested by Mar-

zulli & Maibach (1970), this has the disadvantage of diluting the amount of oil per unit area. However, in view of the fact that it takes longer for the 50 % aqueous vehicle to dry on the skin, it would seem on balance preferable to use undiluted ethanol as the vehicle in routine open photopatch testing.

Contact with psoralens, whether accidental or not, e.g. as in the production of giant hogweed dermatitis or Berloque dermatitis, or in photochemotherapy or dermatoses such as psoriasis, will involve a number of variables which may affect the ability of the skin to respond. These variables include the size and site of the skin area involved, the interval between the application of the photoactive substance and the subsequent irradiation; the number of repeated treatments to the same areas as occurs for example in photochemotherapy or the frequency of applications of perfume. Also, hydration of the skin which may result from sweating or swimming along with genetic factors such as the basic amount of skin pigmentation and ability to tan may well affect the response.

Within the limits of 1-3 square cm our results show that the size of the area tested makes little difference to the reaction obtained. This finding is similar to that of Olson et al. (1973) for sunburn erythema. As in the case of the sunburn reaction, differences may well be found if very small areas, around 1 sq mm, were tested instead. However, the site of skin testing significantly affects the degree of response to a bergamot oil preparation.

Previously, Marzulli & Maibach (1970) did show that the skin of the forearm is less reactive to photopatch testing with bergamot oil than that of the neck or scrotum, both of which areas would appear to have more permeable skin. Our results reveal regional differences in reactivity for different areas of the back, demonstrating the

importance of using comparable test areas in this type of investigation.

It has been established that there is an optimal time interval between the application of psoralen and the subsequent irradiation in the production of the maximal phototoxic effect. Pathak & Fitzpatrick (1959) found this interval to be 30–45 min when different psoralen compounds were tested against guinea pig and human skin. Recently, Arora & Willis (1976) found that for time intervals up to 75 min there was a progressive increase in incidence and intensity of reactions obtained. In our study, the results obtained with bergamot oil and 8-MOP show that intervals of 1 and 2 h between application and irradiation yield maximal response. Concentrations of up to 20 % bergamot oil in ethanol produced no reactions when irradiation immediately followed the application; 5 % bergamot oil in PMF on the other hand produced positive responses in the two subjects tested. This result suggests that when the vehicle used facilitates penetration of the psoralen through the stratum corneum, positive responses may be obtained after very short periods of time. This conclusion is supported by the fact that positive responses to only 2.5 % bergamot oil in ethanol were obtained when the stratum corneum was removed prior to photopatch testing with an '0' time interval between the application and the subsequent irradiation. The finding that the horny layer is the major barrier to penetration of psoralens was suggested by Kammerau et al. (1976) who showed that 70–90 % of topically applied, radioactively labelled 8-MOP in four standard vehicles, did not enter the horny layer and was finally lost through sloughing. The flow of the substance through the horny layer was dependent on the vehicle used. This conclusion was also apparent in the work of Kaidbey & Kligman (1974) who showed that photosensitizing potential of 8-MOP is

strongly influenced by the choice of vehicle.

The duration of phototoxicity due to psoralens after topical application is obviously dependent on the concentration applied. With high concentrations, the interval between application and irradiation may be extended and a positive response will still be obtained. An interesting aspect of our results is that this duration may also depend on the vehicle used; PMF which results in increased penetration through the horny layer, produces a shorter period in which phototoxicity persists than does ethanol, and the possibility that an ethanol vehicle produces some kind of reservoir effect should be considered.

The studies of Burdick (1966), Kligman & Goldstein (1973) and Arora & Willis (1976) show that phototoxic reactions with psoralens have an initial latent period of some 24 h, the intensity reaching a peak at 72–96 h. Our experiments with bergamot oil and 8-MOP demonstrate that mild reactions may not appear until the third day after exposure and on a number of occasions only on the 4th day. The majority of strong reactions show some erythema on the first day which progresses to oedema and sometimes bulla formation on the third and fourth days.

The effect of repeated photopatch testing at the same site with both 5-MOP and 8-MOP was striking and the decrease in MEC produced was approximately proportional to the repeated number of photopatch tests. This has a direct bearing on the photochemotherapy of dermatoses in which psoralens are applied topically. A cumulative effect of repeated treatment is also observed with oral psoralen and UVA (Wolff 1976, Parrish 1976). However, experience with photochemotherapy for psoriasis suggests, presumably due to the protective action of the melanin pigmentation induced by the treatment, that adjustments in dos-

age have to be made in an upward direction to maintain the desired effect of the therapy.

The intensity of human skin reactions to ultraviolet radiation is increased by occlusion prior to irradiation (Cattano 1970). Hydration by water immersion, the application of wet packs and a high environmental humidity produce the same effect in hairless mice (Owens et al. 1975). Occlusion and hydration of the skin also increase the penetration of substances applied topically, and Harber & Baer (1965) and Levine & Harber (1969) reported an increased response to topical but not oral 8-MOP in guinea pigs when the animals were exposed to a humid environment. Similar effects were produced in our study using occlusion and bathing and this could be due to increased absorption of the psoralen as well as increased transmission of the UVA through the stratum corneum. This effect has obvious relevance to the incidence of giant hogweed dermatitis which is usually most severe when contact with the plant occurs on a hot sunny day. Similarly, with the widespread use of perfume materials in sunscreens and other cosmetic accompaniments of summer relaxation, where no effect might normally be expected, a minimal phototoxic reaction may well occur.

The protective function of melanin pigmentation against the injurious effects of ultraviolet radiation is well established (Miescher 1932, Olson et al. 1973, Fitzpatrick et al. 1974). Phototoxic reactions due to pitch (Crowe et al. 1961) and those occurring among celery harvesters (Birmingham et al. 1961) are less common among darker skinned people, and Zurhelle (1928) reported that Berloque dermatitis was more marked in blondes and less so in darker individuals. Carter et al. (1973) have shown that melanin protects against the phototoxic effects of psoralens but only when the pigment is present in a scattered

distribution in the stratum corneum. Such a finding might explain our results when the reactions of various skin colours, whether intrinsic or due to suntan, are compared. There is no difference in reaction when fair and light brown skins are compared and the melanin pigment in these types is found mostly in the lower levels of the epidermis. In our dark skinned subjects and in the suntanned skin where melanin is present in high concentration in the horny layer, we find a significant decrease in reaction level. An additional factor may obtain in the case of suntanned skin. Here there is a change also in the nature of the horny layer itself which may affect the penetration of both the psoralen and the UVA.

The finding that there is no significant difference in phototoxic reaction levels between fair skinned and sallow skinned individuals or between subjects with blue, green or grey eyes and those with similar skin colour but brown eyes, appears to differ from the results of similar comparisons for reactions to sunburn radiation (Pathak et al. 1976) which are more severe in lighter skinned, pale eye colour subjects. This is perhaps not surprising because the penetration into the skin of the shorter wavelength radiation responsible for the latter reaction is more likely affected by a lesser degree of pigmentation.

There is little reason to suppose that the lower thoracic region of the back will vary much between the sexes or with age for skin reactions to phototoxic agents. Our results confirm this negative supposition.

In conclusion, as a result of these studies we recommend the use of the open photopatch test technique for assessing the reactivity of patients with psoralen-induced reactions to sunlight, e.g. in melanoderma in which oil of bergamot is suspected of playing a role. The tests should always be performed in the same general area of skin, preferably the lower thoracic region of the

back, and the patient should avoid factors which lead to skin hydration, such as bathing and sweating. The presence of suntan at the test site and the degree of natural pigmentation should be taken into consideration during the assessment of the results because these tend to decrease the phototoxic response to psoralens.

Although this study was set up originally to determine the most appropriate regime for assessing the phototoxic reactions associated with the application to the skin of oil of bergamot as a perfume ingredient of cosmetics, etc., in the study of photosensitivity reactions of the skin and of pigmentary skin anomalies, the results have a relevance to photochemotherapy in the treatment of psoriasis and other dermatoses. Although the most appropriate ratio of topical to oral administration of the psoralen has yet to be worked out, it seems likely that topical therapy will be used (Lakshmipathi et al. 1976) and therefore the variable factors described in this report will require consideration so as to produce the best therapeutic effect.

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