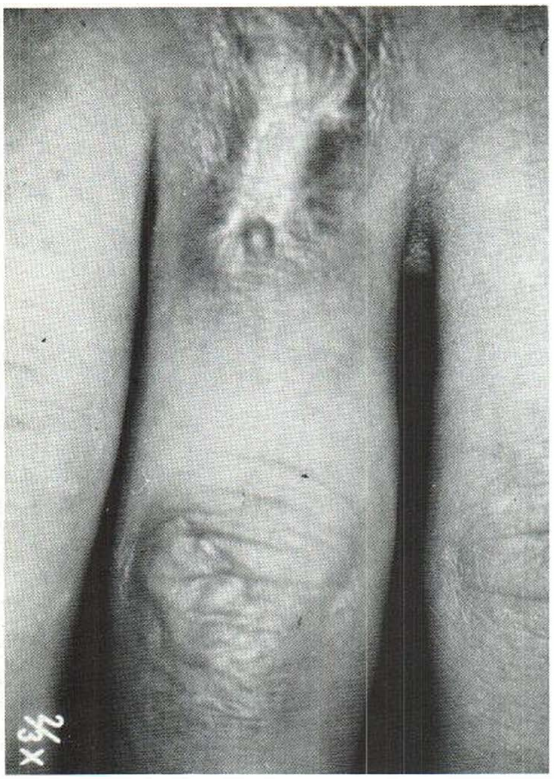

Fig. 2. Same area as in Fig. 1, following 6 weeks of therapy with co-trimoxazole.

marinum infection (1–4, 6, 9, 10). Usually the patients report a history of a preceding minor trauma. Our patient did not remember any damage to the skin, but some kind of minor abrasion on the hands or fingers of a 12-year-old boy would not seem unlikely.

M. marinum may be isolated from the lesion as in our case, from contaminated water, or from walls of swimming pools or aquariums (1, 3, 10). The most suitable culture source, however, seems to be dead or diseased fish (3, 9). Unfortunately no dead fish were available in our case. The history of diseased fish, however, strongly supports that the aquarium was the source of infection.

Most strains of *M. marinum* isolated from infected humans have been relatively resistant to the conventional antituberculous agents, whereas our isolate was not. A remarkable clinical effect of treatment with co-trimoxazole was first noted by Barrow & Hewitt (2) and later confirmed by others (3, 10). On the other hand, *M. marinum* resistant to co-trimoxazole by *in vitro* testing has also been reported (4). Even if human *M. marinum* infections are said to be self-limiting (7), we feel that the prompt response to co-trimoxazole in our patient differs markedly from the usual slow course of spontaneous remission.

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In vitro Studies on the Antifungal Effect of PUVA

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Abstract. The effect of PUVA therapy upon 12 dermatophyte strains, 3 of *Candida albicans*, and 4 of mould fungi was evaluated by the agar dilution method on

Table 1. *Fungus species treated with or without 8-MOP, 30 μg/ml in Sabouraud agar and with or without UVA, 4 Joule/cm²*

TM = *Trichophyton mentagrophytes*, TR = *Trichophyton rubrum*, MC = *Microsporum canis*, EF = *Epidermophyton floccosum*, *C. alb.* = *Candida albicans*

	−UVA		+UVA	
Fungus species	−8-MOP	+8-MOP	−8-MOP	+8-MOP
TM	++++	++	++++	−
TM	++++	+	++++	−
TM	++++	++	++	−
TR	++++	−	−	−
TR	++++	−	++	−
MC	++++	−	++++	−
MC	++++	+	++	−
MC	++++	+	++++	−
MC	++++	−	++	−
EF	++++	−	++++	−
EF	++++	−	++++	−
EF	++++	−	++++	−
C. alb.	++++	++++	++++	−
C. alb.	++++	++++	++++	−
C. alb.	++++	++++	++++	−
Asperg. terreus	++++	++++	++++	−
Scopul. brevic.	++++	++++	++++	+
Acremonium sp.	++++	++++	++++	+++
Asperg. sp.	++++	++++	++++	+

Sabouraud agar, 8-MOP, 30 μg/ml medium plus UVA, 4 Joule/cm² proved to be fungicidal dose to all the strains studied, with the exception of three of the mould fungi. For dematophytes and *Candida albicans* the MIC was between 1 and 3 μg, 8-MOP plus UVA, 4 J; and 3 μg when the UVA dose was reduced to 1–2 J. For mould fungi it was higher. The MFC varied within the range 2 to 30 μg, when the UVA dose was fixed at 4 J. It reached 16 J when the dose of 8-MOP was kept at 4 μg. The wide range in the MFC of the dermatophytes could be due to resistant colonies. 8-MOP alone had a distinctly inhibitory effect upon dermatophytes, but not upon *Candida albicans* or mould fungi.

Key words: PUVA treatment; Antifungal effect of PUVA

From Smith's (11) survey on the effect of radiation upon fungi it is apparent that the inhibitory effect of sunlight has been known since 1821.

Gomez-Vega (5) reported in 1936 that UV of a wavelength exceeding 315 nm had no effect upon yeast fungi or dermatophytes unless they had been pre-sensitized by methyl thionine chloride or Mercurochrome.

Daniels (3) described in 1965, a method for demonstrating phototoxic agents by means of *Candia albicans*. In that study he was the first to demonstrate the antimycotic action of the combination furocoumarins and UVA.

During the subsequent years, few cytological studies on the effect of 8-methoxypsoralen (8-MOP) plus UVA upon yeast fungi saw the light of day (1, 10, 12).

PUVA therapy of psoriasis and certain other diseases focused increased interest upon the antimicrobial effect. So far, however, Oberste-Lehn & Plempel (9) seem to be the only ones to have studied this effect upon a wide spectrum of microorganisms, including dermatophytes.

In the present study the *in vitro* effect of PUVA was tested against dermatophytes, *Candida albicans*, and mould fungi.

Preliminary results of this study have been published previously (7).

MATERIALS AND METHODS

Plates of Sabouraud glucose agar with penicillin, streptomycin, and 8-MOP (Meladinine; Pharma-medica) added were seeded with two drops of a fungus suspension prepared as follows: ¼ cm² material from a Sabouraud agar culture was suspended in 2 ml 0.9% NaCl, homogenized by shaking with 6 glass beads for 10 minutes, and diluted with 2 ml 0.9% saline.

Immediately thereafter plates were irradiated with UVA (Psoralight; Pharma-medica) and uncubated at 26°C.

The readings were graded as follows: ++++: 101–200

Table II. *Fungus species treated with or without 8-MOP, 0.3, 1, 2 or 3 μg/ml in Sabouraud agar and with or without UVA, 4 Joule/cm²*

The figures in parentheses indicate numbers of colonies

Fungus species	−UVA					+UVA				
	−8-MOP	+8-MOP				−8-MOP	+8-MOP			
		0.3	1	2	3 μg/ml		0.3	1	2	3 μg/ml
TM	++++	++++	++++	++++	++++	++++	++++	++++	++	+ (15)
TM	++++		++++	++++	++++	++++		++++	+++	++
TM	++++		++++	++++	++++	++++		++++	+ (12)	+ (1)
TR	++++		+++	++++	+++	++++		−	−	−
TR	++++		++++	++++	++++	++++		+ (1)	−	−
TR	++++	++++	++	+++	++	++	+++	+	−	−
MC	++++		++++	++++	++++	++++		+	+	−
MC	++++		++++	++++	++++	+		+ (18)	−	+ (2)
MC	+++		++++	+++	++	++++		+ (4)	+ (11)	+ (1)
EF	++++		++++	++++	++++	++++		++	+ (9)	+ (4)
EF	++++		++++	++++	++++	++++		+ (5)	+ (2)	+ (3)
EF	++++		++++	++++	++++	++++		+	+	+
C. alb.	++++		++++	++++	++++	++++		++++	++++	+
C. alb.	++++	++++	++++	++++	++++	++++	+++	+	+	+
C. alb.	++++		++++	++++	++++	++++		++++	++	+
Asperg. terreus	++++	++++			++++	++++	++++			++++
Scopul. brevic.	++++	++++			++++	++++	++++			++
Acremonium sp.	++++	++++			++++	++++	++++			++++
Asperg. sp.	++++	++++			++++	++++	++++			++++

colonies. +++: 51–100 colonies. ++: 26–50 colonies. +: 1–25 colonies.—: no growth.

The minimal inhibitory concentration (MIC) was defined as the dose of 8-MOP + UVA which reduced the number of colonies by at least one-half as compared with the control.

The minimal fungicidal concentration (MFC) was defined as the lowest dose which prevented growth completely.

Experiment I

12 strains of dermatophytes, 3 strains of *Candida albicans*, and 4 strains of mould fungi were seeded on Sabouraud plates with and without 8-MOP, 30 μg/ml medium.

For each strain one plate with 8-MOP and one control plate were irradiated with UVA, 4 J/cm², while one plate with 8-MOP and one control plate were not irradiated.

Experiment II

12 strains of dermatophytes, 3 strains of *Candida albicans*, and 4 strains of mould fungi were seeded on Sabouraud plates with and without 8-MOP, 1–2–3 μg/ml medium. Two strains of dermatophytes, 1 of *Candida albicans*, and the 4 mould fungi were also tested with 8-MOP, 0.3 μg/ml. For each strain and each concentration of 8-MOP one plate with 8-MOP and one control plate were irradiated with UVA, 4 J/cm², while one plate with 8-MOP and one control plate were not irradiated.

Experiment III

12 strains of dermatophytes and 3 strains of *Candida albicans* were seeded on Sabouraud agar plates with and without 8-MOP, 3 μg/ml. For each strain one plate with 8-MOP and one control plate without 8-MOP were irradiated with UVA, 1–2–4–8 and 16 J/cm².

RESULTS

From Table I it is apparent that 8-MOP, 30 μg/ml + UVA, 4 J was a fungicidal dose to all the strains tested except for three of the mould fungi which were merely inhibited.

The results that can be deduced from Tables I, II and III may be summed up as follows. For dermatophytes and *Candida albicans* the MIC was between 1 and 3 μg, 8-MOP plus UVA, 4 J and 3 μg when the UVA dose was reduced to 1–2 J. For mould fungi it was higher.

The MFC varied within the range 2 to 30 μg when the UVA dose was fixed at 4 J. It reached to 16 J when the dose of 8-MOP was kept at 4 μg.

Non-fungicidal doses often entailed a 2–4-day delay of appearance as compared with the control.

The irradiation did not result in any striking changes in fungal morphology.

Figures in parentheses indicate that the differences with respect to MFC for the dermatophytes could have been due to a very few resistant colonies.

Table III. *Fungus species treated with or without 8-MOP, 3 μg/ml in Sabouraud agar and with or without UVA, 1, 2, 4, 8 or 16 Joule/cm²*

Figures in parentheses indicate numbers of colonies

Fungus species	−8-MOP +UVA					+8-MOP +UVA				
	1	2	4	8	16	1	2	4	8	16 J
TM	++++	++++	++++	++++	++++	++	+ (20)	+ (8)		−
TM	++++	++++	++++	++++	++++	++++	+++	+++	+ (3)	−
TM	++++	++++	++++	++++	++++	++++	+ (4)	+ (1)	−	−
TR	++++	++++	+++	+++	+	+ (2)	−	−	−	−
TR	++++	++++	++++	++++	+	+ (25)	−	−	−	−
TR	++++	++++	++++	++++	+	++++	−	+ (2)	−	−
MC	++++	++++	++++	++++	−	+	+ (2)	−	−	−
MC	++++	++++	+	+	+	++++	+ (2)	+ (2)	−	−
MC	+++	++++	++++	++	+	+	+ (3)	+ (1)	−	−
EF	++++	++++	++++	++++	+++	++	+ (15)	+ (4)	−	−
EF	++++	++++	++++	++++	++	++	+ (8)	+ (3)	+ (1)	−
EF	++++	++++	++++	+	+	+	+	+ (6)	−	−
C. alb.	++++	++++	++++	++++	++++	++	+	+	+	+
C. alb.	++++	++++	++++	++++	++++	++++	+	+	+	+
C. alb.	++++	++++	++++	++++	++++	++++	+	+	+	+

As is apparent from Table I, 8-MOP alone had a distinctly inhibitory effect upon dermatophytes when the dose was as high as 30 μg/ml, while *Candida albicans* and mould fungi remained entirely unaffected.

The same applied to UVA, but less constantly and less markedly, when the dose was raised to 8–16 J.

DISCUSSION

Owing to variations in spore size, the presence of microconidia, macroconidia and mycelia, and the tendency to clumping of spores, it is impossible to prepare completely uniform suspensions of fungi (5, 6), and this influences the results of the PUVA therapy.

However, method control on experiment III by *Trichophyton rubrum* and *Trichophyton mentagrophytes* strains seeded in triplo showed such good agreement that the MIC was the same within each set, while a one-step difference in dose level occurred for MFC.

As is apparent from the tables the mould fungi were most resistant, while for the other strains the MIC and MFC varied within fairly narrow limits. It will be seen that differences with regard to MFC could be due to a very few resistant colonies.

The reaction between furocoumarins and the pyrimidine bases of DNA caused by irradiation at 365 nm may be compared to the effect of 260 nm (8).

There is a similarity also between the survival curves (a "shoulder" followed by a rectilinear part) for *Trichophyton mentagrophytes* following irradiation with UVC (6) and the corresponding curves following treatment of *Saccharomyces* with 8-MOP and UVA (8).

In studies on the effect of UVC upon *Trichophyton mentagrophytes*, Emmons & Hollaender (4) induced a temporary delay in appearance, hyphal changes, and—signifying a mutagenic effect—an increased tendency to the production of variants.

No such investigations appear to have been performed for PUVA, but according to Averbeck & Moustacchi (1) 8-MOP + 365 nm is less effective than 254 nm for inducing cytoplasmic "petite" mutation in yeast fungi.

In the present investigation we did not observe any macroscopic changes in the surviving fungi.

Gomez-Vega (5) could cure nail fungi by mercurochrome plus sunlight through a lens.

In psoriatics and other patients under PUVA therapy there is a possibility that a co-existing fungal disease may be cured.

Incidentally, the possible therapeutic utilization of PUVA therapy must be weighed against the attendant risk.

The weaker antimycotic 8-MOP alone, can hardly according to the findings of others (2, 9), compete with the numerous other antimycotic agents on the market.

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Effect of UV-B Erythema of Psoralen, Trimethylpsoralen, and 8 Methoxypsoralen[1]

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Abstract. The MED for UV-B was measured 2 h and 24 h after oral intake of Psoralen (P) 0.49 mg/kg in 10 healthy volunteers. MED was increased when UV exposure had taken place 2 h after intake. The same increase was observed after 0.24 mg/kg and 0.98 mg/kg, with a non-significant dose dependence. This effect was not detectable when UV exposure had taken place 24 h after oral intake of the drug. In a second experiment the effects of oral intake of both trimethylpsoralen (TMP) and 8 Methoxypsoralen (8 MOP) mg/kg, were assessed in 29 subjects. While TMP increased MED when taken 2 h before UV exposure ($p<0.001$), 8 MOP had the opposite effect ($p<0.01$). TMP effect was observed mainly in fair-haired people ($p<0.05$) and 8 MOP effect mainly in females ($p<0.05$).

Key words: Trimethylpsoralen; 8 Methoxypsoralen; Psoralen; UV erythema; UV-B

In a previous paper (1) we have shown that trimethylpsoralen (TMP) significantly increased the MED for UV-B during the few hours following oral intake. This effect was dose dependent and probably related to the absorption of UV-B by the drug while it was present in the skin. In order to know whether this property belongs to TMP alone or to all of the psoralen group, similar experiments were conducted with Psoralen (P), 8 methoxypsoralen (8 MOP), and again with Trimethylpsoralen (TMP). The protective effect of TMP against UV-B erythema was confirmed. A similar effect of P was found while, on the contrary, 8 MOP displayed a sensitizing action.

MATERIALS AND METHODS

The UV-B source was a 150 W XBO high-pressure xenon lamp. The spectrum had been adjusted to the sun spec-

Abbreviations: MED=minimal erythemal dose; UV-B=ultraviolet B rays

[1] Presented in part at the meeting of the French Dermatological Society, Biological Session, Paris 6-8-78, and at the second European Workshop on Mammalian Melanin Pigmentation, London 21 and 22-11-79.