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International Journal of Pharmaceutics 133 (1996) 245–252

international journal of pharmaceutics

Percutaneous absorption of three psoralens commonly used in therapy: effect of skin occlusion (in vitro study)[1]

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Received 29 September 1995; revised 2 January 1996; accepted 12 January 1996

Abstract

Occlusion is recognised as an enhancer of percutaneous absorption. Some authors suggested that this effect is related to the molecular polarities of applied drugs. To verify this hypothesis, an in vitro study was performed to assess a topical absorption, under occlusion, in relation to the molecular polarity of three furanocoumarins: 5-methoxypsoralen (5-MOP), 8-methoxypsoralen (8-MOP) and trimethylpsoralen (TMP). These compounds present the same basic chemical structure but have different degrees of lipophilicity. They are commonly used, orally or topically, in conjunction with UVA irradiations to treat skin diseases such as psoriasis and vitiligo. Ethanolic solutions of the psoralens were deposited onto human abdominal skin fragments (6.3 $\mu g/cm^2$), mounted on Franz® diffusion cells ($n = 12$), which were maintained at 37°C. Occlusion was retained by placing rubber corks on the top of Franz® cells ($n = 6$). The receptor fluid was constituted with 1.4% of human serum albumin solution. At each sample point (2, 4, 6, 8, 12 and 24 h), the entire content of the receptor chamber was removed and replaced by fresh albumin solution. Psoralens quantities in the removed solutions were determined by HPLC. The lipophilicity of the psoralens was established via the partition coefficient in an octanol/water system: TMP > 5-MOP > 8-MOP. The outcome of the results illustrates that occlusion does not always have an enhancing effect in percutaneous absorption. Occlusion impact is influenced by the polarity of the drug. It increases the absorption of moderately lipophilic molecules (i.e. 8-MOP, 5-MOP) but is almost ineffective on the absorption rate of molecules highly lipophilic (i.e. TMP).

Keywords: Occlusion; Percutaneous Absorption; Human Skin; Psoralens; Lipophilicity

1. Introduction

Some in vitro and in vivo studies (Anderson et al., 1973; Rietschel and Devillez, 1981; Schaefer et al., 1982; Hartmann, 1983; Ryatt et al., 1988)

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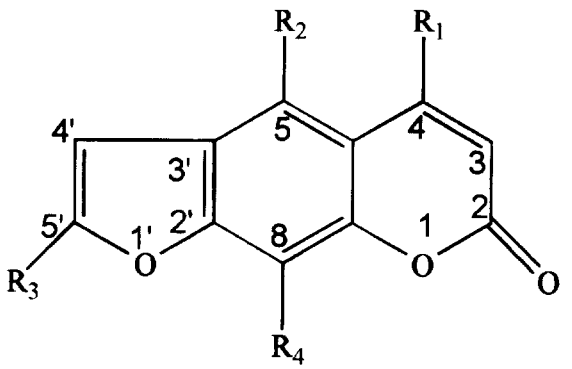
[1] Part of this study was presented at " The Fourth International Prediction of Percutaneous Penetration Conference", 18–22 April 1995, La Grande Motte, France.

PII S0378-5173(95)04451-1



$R_1 = R_2 = R_3 = R_4 = H$	Psoralen (P)	MW = 186
$R_1 = R_2 = R_3 = H$; $R_4 = OCH_3$	8-methoxypsoralen (8-MOP)	MW = 216
$R_1 = R_3 = R_4 = H$; $R_2 = OCH_3$	5-methoxypsoralen (5-MOP)	MW = 216
$R_2 = H$; $R_1 = R_3 = R_4 = CH_3$	4,5',8-trimethylpsoralen (TMP)	MW = 228

Fig. 1. Molecular structures and molecular weights (MW) of psoralen ring and its three derivatives, studied in this work.

indicated variable effects of skin occlusion on percutaneous absorption. The results of these researches had demonstrated a number of factors which could influence the cutaneous absorption; namely, the modality of occlusion, the type of solvents, the skin temperature, the molecular polarity, etc. The last factor is probably of a major importance (Bucks et al., 1989; Treffel et al., 1992). However, in these studies, the basic molecular structures of employed compounds were different and, consequently, the observed changes could have been related to other factors.

The aim of this work was to determine, in vitro, the effect of occlusion on drugs percutaneous absorption in respect to their molecular polarity. To carry out this work, we compared the skin absorption of three psoralens: 5-methoxypsoralen (5MOP), 8-methoxypsoralen (8-MOP) and 4, 5', 8 trimethylpsoralen (TMP). Furocoumarin is their basic chemical structure (Fig. 1). These compounds are currently employed in dermatology, orally or topically, associated with Ultraviolet A (UVA) irradiation to treat skin diseases such as psoriasis and vitiligo. The combination of the two previous elements is known as PUVA therapy.

For optimizing the PUVA therapy, after topical application of psoralens, we need to know the kinetic of drugs penetration under different clinical situations. The disclose of the molecular polarity impact on skin absorption in relation to occlusion would help to find which of these psoralens could better match the therapeutic requirements.

2. Materials and methods

2.1. Skin preparation

Immediately after surgical operation (women abdominoplasties), the skin was frozen at − 18°C. A day before the experiments, the skin was placed

in a refrigerator at +4°C. The subcutis lipid layers were removed, 1 h before the experiments, and the skin was cut into (3 × 3) cm^2 pieces and randomized: the original disposition of skin fragments on the body was not considered. The skin employed, in this study, was from a 43-year-old female subject.

2.2. Chemicals

5-MOP was kindly given by Bergaderm Laboratories (Rungis - France), 8-MOP and TMP were purchased from Sigma chemical company (France).

2.3. Experiments

Skin pieces were fixed on Franz® diffusion cells (Type FDC 200, Sommerville, NJ, USA). The absorption surface area was 3.14 cm^2. On the upper layer of the skin, the *stratum corneum*, psoralens ethanolic solutions were deposited. The 8 ml volume of the lower chamber of Franz® cell was filled with a receiving human albumin, diluted in bidistilled water (1.4%). This concentration is close to that of the interstitial fluid of the skin (Vermeer et al., 1975). The lower chambers of diffusion cells were surrounded with a water bath maintained throughout the experiment at 37°C, corresponding to the normal skin temperature. The recipient liquid was continuously agitated with a magnetic stirrer at a rotation speed of 400 rpm.

For each psoralen compound, 12 Franz® diffusion cells were employed: 6 cells without occlusion and 6 cells with occlusion.

The ethanolic solution (50 μl: 6.3 $\mu g/cm^2$) of TMP or 5-MOP or 8-MOP of equal concentration (400 μg/ml) was deposited, with a Hamilton® microsyringe, on each skin fragment. Immediately after a psoralen solution deposit, the ethanol was evaporated by a hair drier to avoid any enhancement of ethanol penetration under occlusion (Bucks et al., 1989) and to diminish its effect on *stratum corneum* cells membranes. Samples from the same abdominal skin were employed for the three psoralens, to prevent an inter-individual variability.

The occlusive system was made of rubber corks which were fixed on the upper chamber of diffusion cells in such a way that the corks did not touch the skin surface. The corks had been pierced in the center to prevent any air pressure increase within the upper chamber. When the corks were in place, their holes were obstructed with cotton. The recipient fluid was removed at different periods of times, 2, 4, 6, 8, 12 and 24 h, after a psoralen deposit.

2.4. Determination of drugs concentrations

At the appropriate hours, aliquots were removed from the receiver cells. The determination of psoralens concentrations was carried out by High Performance Liquid Chromatography (HPLC). After extraction with heptane/dichloromethane (4:1/v:v), the drug in 1 ml of the receptive solution was assessed by using a Merck-Hitachi HPLC apparatus (Model 655 A-II). The mobile phase was constituted of methanol-water (60:40/v:v) and the stationary phase used was Rp18 LiChroCART® 125-4 (Merck, Darmstadt, Germany) (Stolk et al., 1981). A Merck-Hitachi 1000 spectrofluorometer detector was employed to increase the sensitivity determination (Prognon et al., 1983). The excitation (λex) and emission (λem) wavelengths were selected: 314 nm and 490 nm, respectively. For 5-MOP and 8-MOP assay, calibration curve was obtained by adding 0, 5, 10, 15 and 20 μl of the respective psoralens solutions (20 μg/l ethanol) to drug-free human serum albumin samples, with 10 μl of an internal standard in each tube (Muret et al., 1993). The internal standard used was a solution of TMP (20 μg/l ethanol). Peaks height ratios (psoralen to determine/internal standard) were calculated and the concentrations of the drug in receptive solutions were found directly from the standard solution graph. 5-MOP (20μg/l ethanol) was used as an internal standard solution for the determination of TMP.

For statistical analysis, the Mann-Whitney test was used to compare the cumulated psoralens quantities absorbed over 24 h.

Table 1
Comparison of psoralens hours flux *without* and *with* occlusion

Receptor fluid removal hours after the deposit of psoralens solutions	Psoralens flux →					
	Without occlusion			*With* occlusion		
	TMP $\bar{X}$ ± s.e.m	5-MOP $\bar{X}$ ± s.e.m.	8-MOP $\bar{X}$ ± s.e.m	TMP $\bar{X}$ ± s.e.m	5-MOP $\bar{X}$ ± s.e.m	8-MOP $\bar{X}$ ± s.e.m.
2 h	7.06 ± 1.23	7.48 ± 0.44	27.40 ± 2.72	6.06 ± 1.06	9.33 ± 2.63	27.78 ± 4.56
4 h	5.27 ± 0.94	10.31 ± 2.42	39.29 ± 7.60	5.87 ± 0.89	28.80 ± 8.35	36.11 ± 8.50
6 h	5.47 ± 2.17	12.75 ± 1.72	50.70 ± 4.67	4.28 ± 1.34	30.04 ± 7.30	95.51 ± 32.28
8h	4.23 ± 0.93	29.49 ± 4.05	75.06 ± 7.52	4.13 ± 0.38	32.85 ± 4.06	123.83 ± 33.67
12h	2.56 ± 0.39	16.32 ± 5.76	45.4 ± 3.82	3.38 ± 0.42	46.51 ± 11.46	78.64 ± 15.82
24 h	1.59 ± 0.35	10.57 ± 1.66	34.16 ± 3.44	2.80 ± 0.40	24.32 ± 7.15	62.86 ± 7.30

Each value represents a psoralen HPLC determination average results, ($\bar{X}$ ± s.e.m.), of six Franz® cells receptor fluids.
s.e.m., standard error of the mean.
The results are given in ng/cm^2/h.

2.5. Determination of partition coefficients

The partition coefficient was carried out according to the method of Dearden and Bresnen (1988). We performed a water/1-octanol (v/v) mutual saturation for 3 h with mechanical stirring of the mixture. Ethanolic solutions (50 μL) containing 200 μg of psoralen solutes were evaporated in glass-tubes and 2 mL of each saturated solvent was added to the tubes. About 100 inversions, for 5 min of the stoppered tubes, were achieved as recommended by Leo et al. (1971). The phases were separated by centrifugation at 3000 rpm for 10 min. To determine the concentration in each phase, the octanol phase was diluted to 1/100 for 5-MOP, TMP and to 1/20 for 8-MOP. The aqueous phase was diluted (50/50) with pure methanol to ensure good detection (Prognon, 1984). The solutions (n = 10) were determined by using spectrofluorimetric technique with the help of a Shimadzu® RF 540 apparatus. For the measurement, selected excitation wavelengths (λex) were at 302 (8-MOP), 313 (5MOP) and 334 nm (TMP). Observed emission wavelengths (λem) were 500, 480 and 450 nm, respectively. The slitwidths were 10 nm for both excitation and emission beams.

3. Results

3.1. Psoralens cutaneous permeation

Table 1 and Fig. 2 show the averages of psoralens flux in respect of albumin solution removal hours, without (Fig. 2A) and with occlusion (Fig. 2B). The difference of cumulative absorption, without and with occlusion, over 24 h, for each psoralen, is shown in Table 2 and Fig. 3. The results show that there is a significative increase of absorption of 8-MOP and 5-MOP under occlusion, P = 0.032 and 0.007, respectively. The TMP was poorly absorbed, even under occlusion (P = 0.19).

3.2. Lipophilicity determination

The octanol-water partition coefficient (K) was established as a polarity index (Guy and Hadgraft, 1989). It was determined (Table 2) as the concentrations ratio between both equilibrated solvents (octanol and water). The involved concentrations were determined from the measured relative fluorescence intensity. The fluorescence peak was integrated and the concentration was assessed from an external standard calibration curve established for each solute.

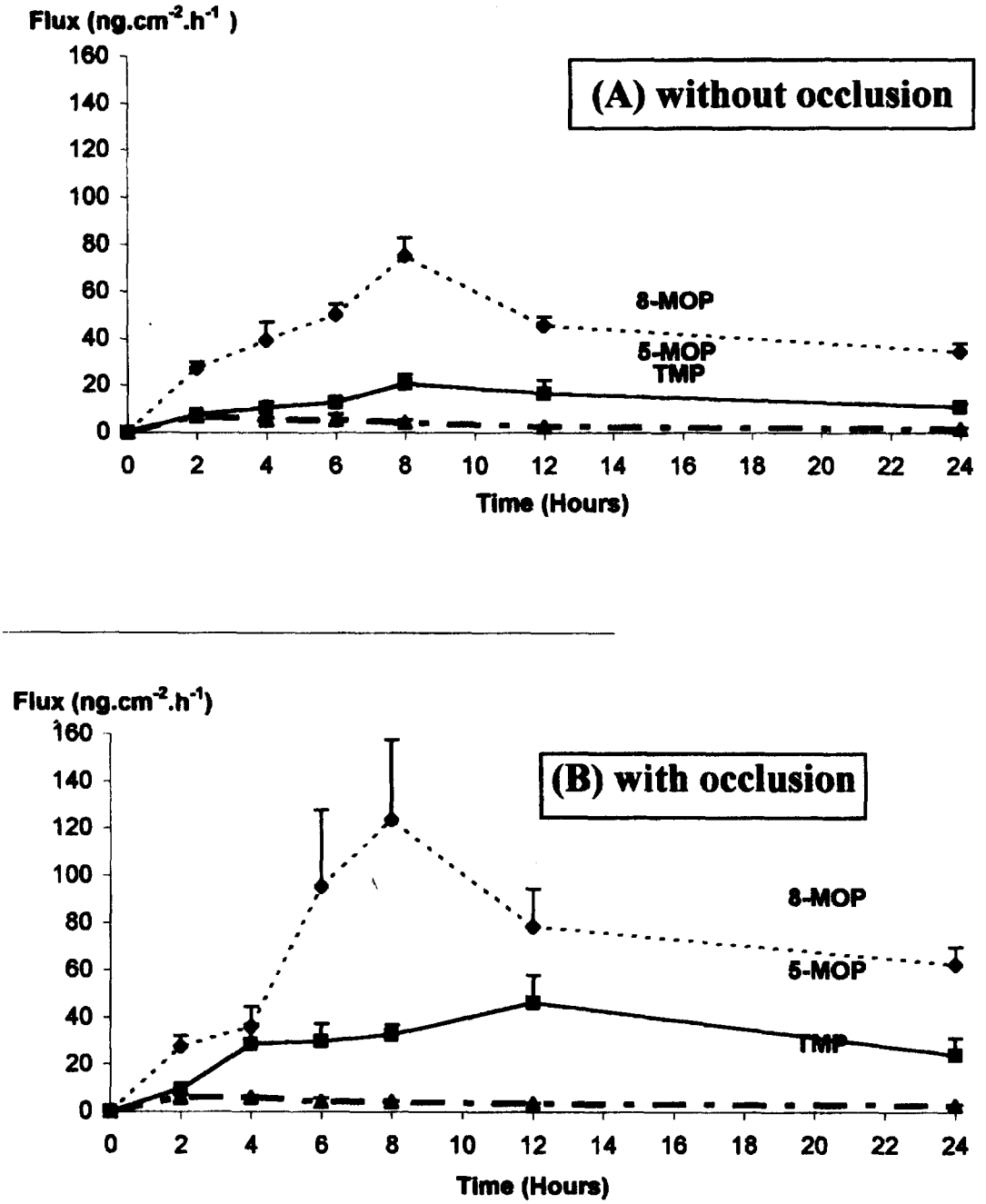


Fig. 2. Psoralens flux in respect of removal of albumin solution which was carried out at different periods of time (2, 4, 6, 8, 12, 24 h). (A) *Without* occlusion; (B) *With* occlusion. T: standard error of the mean (s.e.m.).

Table 2
Cumulative psoralens absorbed (± s.e.m.) over 24 h, *without* and *with* occlusion (columns 3 and 4)

Psoralens	Log of partition coefficient (log K: $\bar{X}$ ± s.d.)	Psoralen absorption *Without* occlusion ng/cm²/24 h $\bar{X}$ ± s.e.m.	Psoralen absorption *With* occlusion ng/cm²/24 h $\bar{X}$ ± s.e.m.	*P*
TMP	3.14 ± 0.01	73.5 ± 12.8	87.9 ± 8.5	0.19
5-MOP	2.00 ± 0.01	294.5 ± 53.3	679.8 ± 164.2	0.007
8-MOP	1.93 ± 0.01	976.5 ± 83.1	1769.8 ± 372	0.032

The log Ks (expression of the lipophilicity), indicated in the second column, show an inverse correlation with cumulative quantities of psoralens.
s.d., standard deviation.
In the fifth column, the significance level (*P*) is indicated, calculated values given by the Mann-Withney test.

The partition coefficient (mean ± s.d.) determination demonstrated that the TMP is the most lipophilic (log K = 3.14 ± 0.01), followed by the 5-MOP (log K = 2.00 ± 0.01) and 8-MOP (log K = 1.93 ± 0.01) (Table 2).

4. Discussion

In this study, we compared, in vitro, the permeation through human skin of three psoralens commonly used in therapy. The aim of this research was to verify the effect of occlusion on skin absorption in respect of the molecular polarity. The investigated psoralens have the same basic molecular structure (Fig. 1), but their lipophilicities are different: TMP > 5-MOP > 8-MOP (Table 2). This order of hydrophobicity was already indicated in the literature (Prognon, 1984).

The results of this work demonstrated that occlusion enhanced absorption of two psoralens (8-MOP and 5-MOP) which have moderate lipophilic character (Table 2 and Fig. 3). The average flux of psoralens displayed a curve of finite dosage type (Fig. 2A and B). The TMP which presents the highest lipophilicity (log K = 3.14 ± 0.01) was weakly absorbed and occlusion did not modify much this situation (Table 2 and Fig. 2 and 3). A possible explanation of TMP weak absorption could be that the hydrophobic *stratum corneum* has more affinity for molecules which have high lipophilicity.

The general conception was that occlusion enhances cutaneous absorption (Mc Kenzie and Stoughton, 1962). Some authors indicated that occlusion increases molecular percutaneous absorption as it incites to hyperhydration and to a rise of temperature (Fritsch and Stoughton, 1963; Schaefer et al., 1982; Berardesca and Maibach, 1988). Others suggested that molecular polarity could be one of the influencing parameters to consider in absorption under occlusion (Mc Kenzie, 1962; Bucks et al., 1989; Treffel et al., 1992). It seems that occlusion has no or slight effect on amphiphilic molecules. The caffeine, an amphiphilic molecule, diffuses easily accross the hydrolipidic *stratum corneum* layers, as demonstrated, in vitro, by Bucks et al. (1989) and Treffel et al. (1992). In the case of molecules having moderate lipophilic character, the occlusion increases the percutaneous absorption, as demonstrated with the citropten by Treffel et al. (1992). These data are confirmed by our results for 8-MOP and 5-MOP. In addition, our experiments have demonstrated that occlusion has no or little effect on very pronounced lipophilic molecules percutaneous absorption, as in the case of TMP.

Our data indicate that 8-MOP had a good permeation without and with occlusion and its passage across the skin layers was less influenced by occlusion than the 5-MOP (1.7 vs. 1.2 folds) (Table 2 and Fig. 3). There is an inverse correlation between these cumulative values and the partition coefficients of the three molecules (Table 2). The TMP (the most lipophilic molecule) badly and slowly crossed the skin layers in the two conditions (without and with occlusion). The *stra-*

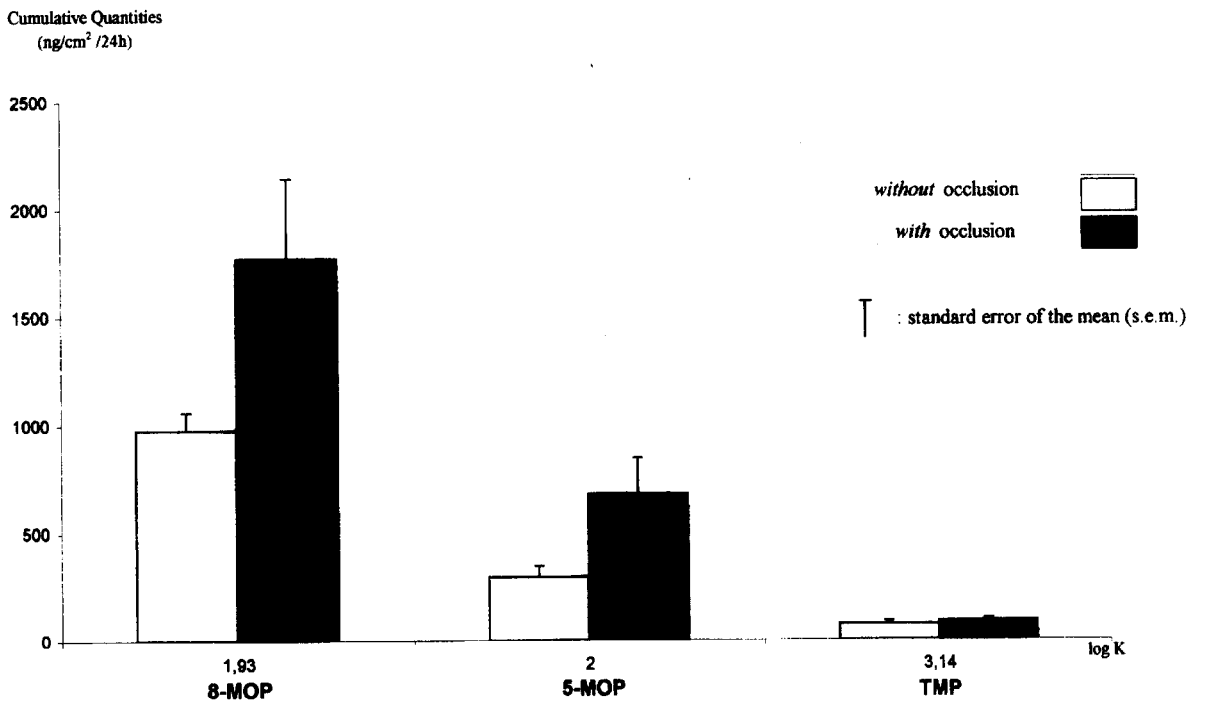


Fig. 3. Cumulated absorbed quantities over 24 h, after psoralens ethanolic solutions deposit on skin surface fragments. The histogrammes represent the average values ± s.e.m. for each psoralen, *without* and *with* occlusion; log Ks give the correponding lipophilicity of each molecule.

tum corneum retention of TMP makes this molecule very useful in PUVA therapy as it stays a long time in the epidermis layers. For dermatological diseases, the skin is the principal target to treat, and it is always hoped that psoralens pass in small concentrations in blood because of their toxicity. The TMP is suitable (without and with occlusion) to treat, topically, skin diseases such as vitiligo and psoriasis. It could be used in PUVA bath therapy as hydration did not deplace it from the epidermis, where it is well fixed.

Acknowledgements

We are thankful to Mrs Micheline Chettouh and to Miss Valérie Mathez (Faculté de Médecine et Pharmacie) for their technical assistance.

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