

Photoepicutaneous testing with the chamber method

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The chamber test, so far successfully applied for epicutaneous testing, proved to be suitable for photoepicutaneous testing as well. A simple and reliable method was developed by dividing the test areas (after removing the tests from the skin) into two equal parts, one for ultraviolet irradiation and the other for dark control. Variations in the amounts of the test substances had a slight influence on the size but not on the strength of the reactions.

Key words: photodermatosis – skin tests, photoepicutaneous.

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The reactivity of the skin to phototoxic and photoallergic agents is usually studied using various photopatch test procedures (Epstein 1964). A great disadvantage of these methods is the large area needed for double test series, one for ultraviolet irradiation and the other for dark control. We have developed a space-saving method for photoepicutaneous testing based on the chamber test method developed by Pirilä (1975).

Material and Methods

The epicutaneous tests were made with the chamber method (Pirilä 1975) (Finn Chamber from Epitest Ltd., Fredrikinkatu 61, SF 00100 Helsinki 10).

The test substances were methoxsalen (8-methoxypsoralen) at 0.1% and crude coal tar at 5% in petrolatum. The occlusion time for methoxsalen was 2 hours and that for coal tar 24 hours. After the chambers were removed, the test areas were cleaned by pressing several times with blotting paper, avoiding the spread of the test substances. The effect of the amount of test substance

used was examined by placing various amounts of the test substance – 10 μ l, less than half volume, 20 μ l, more than half volume, 40 μ l, overfilled – into the chamber with a Mantoux syringe.

The tests were done in three vertical rows. The first row was irradiated as a whole; one half of the second row was covered with black, lightproof plastic adhesive tape; and the third row was totally covered.

The source of ultraviolet irradiation was an apparatus consisting of 6 Philips TL 20W/08 black light tubes with an emission peak of 365 nm. The tubes were installed at 2 cm intervals. Between the tubes and the patient there was a 3 mm thick plate of silicate glass to remove all radiation under 320 nm (Maibach et al. 1967, Owens et al. 1968). The exposure time was 5 minutes at a distance of 5 cm giving a total of 170 mJ/cm² of ultraviolet irradiation. Plastic-coated carbon paper fixed with porous Scanpor® (Norgesplaster A/S, Oslo) tape served as a lightproof covering during the follow-up time of two days. The reactions were read 24 and 48 hours after the ultraviolet irradiation.

Table 1. Mean reaction indices with 0.1% methoxsalen and 5% coal tar. The amount of the test substances was 20 µl.

	No. of tests	Mean reaction indices			
		ultraviolet irradiation 24 h	dark control	ultraviolet irradiation 48 h	dark control
Methoxsalen 0.1%	45	1.9 ^{*)}	0.0	2.3	0.0
Coal tar 5%	20	1.1	0.2	0.8	0.1

^{*)}

0 = no reaction

1 = erythema

2 = erythema with moderate oedema

3 = oedema with beginning vesiculation

4 = uniform bulla

tion. They were graded on a scale of 0–4, in which 0 = no reaction, 1 = erythema, 2 = erythema with moderate oedema, 3 = oedema with beginning vesiculation and 4 = uniform bulla (Kaidbey & Kligman 1974). In addition, the diameters of the reactions were measured. The subjects were dermatologic patients without any evidence of abnormal reaction to light. Patients under medication with a possible photosensitizing capacity were excluded.

Results

Methoxsalen (0.1% in petrolatum) gave a positive reaction in all the tests with ultraviolet irradiation (Table 1). The mean reaction indices after 24 and 48 hours were 1.9 and 2.3, respectively, the most usual reaction thus being erythema and moderate oedema. On all the test sites the reaction extended to the whole area covered by the chamber. No reactions were seen in the control areas.

The reactions using coal tar were weaker than those elicited by methoxsalen (Table 1). The reactions showed a tendency to disappear after 24 hours. The tar also irritated the skin in 14 out of 60 tests without ultraviolet irradiation. The reactions in all these

cases were, however, markedly milder than those after ultraviolet irradiation. The amount of coal tar did not have any marked effect on the strength of the reactions. As a whole, the mean sizes of the reactions caused by various amounts of coal tar differed only very slightly from each other. On the other hand, the sizes of reactions elicited by methoxsalen increased according to the amount of test substance used, but in no

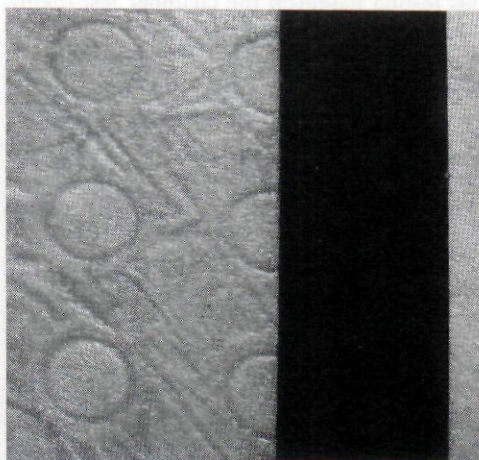


Fig. 1. Before irradiation of the tests, one half of each test area was covered with lightproof tape.

Table 2. The influence of the amount of the test substance on the diameters of photoepicutaneous test reactions.

	No. of cases	Diameters of test reactions					
		10 μ l		20 μ l		40 μ l	
		mean mm	ranges mm	mean mm	ranges mm	mean mm	ranges mm
Methoxsalen 0.1%	45	7	5-9	10	8-12	12	11-14
Coal tar 5%	20	10	8-11	10	8-12	11	10-13

Table 3. Mean reaction indices of the halved (split) tests compared with those of ordinary, non-split photoepicutaneous tests (24 hours after irradiation; the amount of the test substance 20 μ l).

	No. of tests	Mean reaction indices			
		Non-split tests		Split tests	
		ultraviolet irradiation	contr.	ultraviolet irradiation	contr.
Methoxsalen 0.1%	45	1.9 ^{*)}	0.0	1.9	0.0
Coal tar 5%	20	1.1	0.2	1.1	0.2

^{*)}
0 = no reaction
1 = erythema

2 = erythema with moderate oedema
3 = oedema with beginning vesiculation
4 = uniform bulla

case did the reaction extend to the neighbouring reaction (Table 2).

In order to minimize the area needed for tests, the test areas were divided into two equal parts with black, lightproof adhesive tape (Fig. 1). The strength of the reactions of this new method was equal to the strength of the reactions elicited by the ordinary method (Table 3). The borderline between the halves exposed and unexposed to irradiation was well demarcated and sharp, and in no case did the reaction show a tendency to spread into the non-irradiated area (Fig. 2).

Discussion

Methoxsalen and coal tar were chosen for this study because of their well-known

phototoxic properties (Crow et al. 1961, Everett & Miller 1961, Kaidbey & Kligman 1974, Owens et al. 1968). The source of ultraviolet rays was Philips black light tubes, internationally accepted for irradiating photopatch tests.

The distance from the tubes to the patients' skin was 5 cm and the exposure time only 5 minutes. This period is long enough for ordinary purposes, giving a total dose of 170 mJ/cm². If greater doses of ultraviolet irradiation are needed, the exposure time may be extended.

Plastic-coated carbon paper as occlusive material proved to be lightproof, easy to fix on to the skin, non-irritating and inexpensive. Sweat retention was not disturbing, probably because of the use of porous Scan-

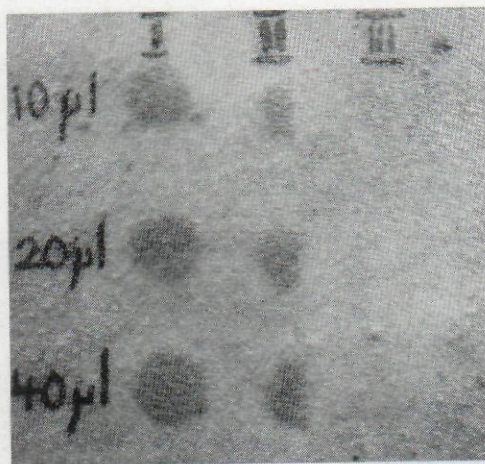


Fig. 2. Phototoxic reactions with methoxsalen 0.1% 48 hours after ultraviolet irradiation.

I=whole test areas irradiated

II=left halves of the test areas irradiated, right halves of the test areas unirradiated (See Fig. 1)

III=unirradiated tests

por® tape as the fixing bandage. The test chambers (aluminium) served as lightproof coverings before the irradiation. Some patch test materials may leak light and are therefore not suitable for photoepicutaneous tests (Epstein 1963, Willis & Kligman 1969).

On the basis of the results of the present study, the Finn Chamber method for epicutaneous tests seems to be suitable for photoepicutaneous testing as well. The results are highly reproducible, and variations in the amounts of the test substances in the chambers did not influence the strength scale of the reactions. For this reason it can be expected that individual variations in the testing technique do not have a significant effect on the test results. Moreover, the test areas can be simply divided into two equal parts with lightproof adhesive tape before ultraviolet irradiation, which makes the extra dark control test un-

necessary. The light-induced reactions of this "fifty-fifty" method did not diminish in strength when compared with those of non-divided tests. With this half-area photo test application of the Finn Chamber method, a large number of tests can be performed on a small area, e.g. tests with ten substances on a 5 cm × 11 cm skin area.

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