

ORAL PHOTOCHEMOTHERAPY IN LICHEN PLANUS (LP) AND MYCOSIS FUNGOIDES (MF): ULTRASTRUCTURAL MODIFICATIONS OF THE INFILTRATING CELLS

J. P. Ortonne, D. Schmitt, A. Alario and J. Thivolet

*Laboratoire de Recherche Dermatologique et Immunologie, Department of Dermatology,
Hôpital Edouard Herriot, Lyon Cedex 2, France*

Abstract. Six patients (5 with mycosis fungoides and 1 with lichen planus) treated with PUVA, were subjected to biopsy of lesional skin before and during oral photochemotherapy. Ultrastructurally, a reduction in the density of cellular infiltrate was observed in the superficial dermis. In the same areas, necrotic cellular changes were observed. PUVA therapy exercises its beneficial effect by direct destruction of these cells.

Oral photochemotherapy, applied to dermatological treatment by Parrish et al. (10) is based on the interaction of a photosensitizing agent, 8-methoxypsoralen and long wave (320-400 nm) ultraviolet light. Such treatment is effective in psoriasis but also in several other dermatoses, particularly lichen planus (9), mycosis fungoides (3, 4, 5, 15). In the two latter, clinical improvement is reflected by disappearance of the dermal infiltrate. At the end of treatment, normal (LP) or proliferating (MF) lymphocytes disappear from the lesion. The mechanism of this disappearance is not known. As the association of psoralens and UVA both in vitro (6) and in vivo (12) induces changes in circulating lymphocytes, we believed that lymphocytes are destroyed directly at the site of benign or malignant cutaneous infiltration as a result of oral photochemotherapy. Consequently, we have studied lesional skin ultrastructurally before and during PUVA therapy in 6 patients with lichen planus or mycosis fungoides.

MATERIAL AND METHODS

Patients

Six patients were included in the study, 5 of whom had infiltrated plaques of mycosis fungoides and one lichen planus. In all cases, diagnosis was confirmed histologically.

Treatment Regime

Light sources

Hexagonal "stand-up" light boxes (cabin DIXERAY, etc. DIXWELL, Lyon, France) containing 84 fluorescent bulbs (bulbs Philips TL 09) mounted vertically were used as UVA sources. These bulbs have a continuous spectrum of high intensity irradiation between 320 and 400 nm, with a peak on average 4 to 4.5 mW/cm² at a distance of 50 cm from the bulbs.

Exposures and dosages

Methoxsalen (meladinine, Laboratories Promedica): ten milligram capsules of methoxsalen were given 2 hours prior to UVA exposure, according to patient weight: 25 kg or less: 10 mg of 8 MOP, 26 to 45 kg: 20 mg of 8 MOP, 46 to 60 kg: 30 mg of 8 MOP, 61 to 75 kg of 8 MOP: 40 mg of 8 MOP, 76 to 90 kg: 50 mg of 8 MOP, greater than 90 kg: 60 mg of 8 MOP.

UVA dosage. Initial exposure times were based on skin type (2). Initial UVA dose was between 1.5 and 2 J/cm² and was increased at the first and third session of each week. The maximum dose administered at the end of treatment never exceeded 7 J/cm² in any single session. The patients were treated three times per week with an interval of 48 hours between each session.

Evaluation of clinical response. The clinical results were recorded as follows:

excellent: total flattening of the lesions
Good: diminution of clinical infiltration of lesions
fair: slight improvement
poor: no improvement

Histomorphological Studies (E.M.)

Punch biopsies were taken in all cases under 2% lignocaine local anaesthesia before treatment, and were repeated at varying intervals after various doses of PUVA (Table I). The specimens were divided for light and electron microscopy. The successive biopsies were always taken from the same lesional plaque in the patients with M.F. A total of 10 biopsies taken during photochemotherapy were examined. For electron microscopy, the biopsy specimens were fixed in 2% glutaraldehyde for 2 hours, post-fixed with osmium tetroxide for 2 hours, dehydrated in alcohol, embedded in Epoxy resin and sec-

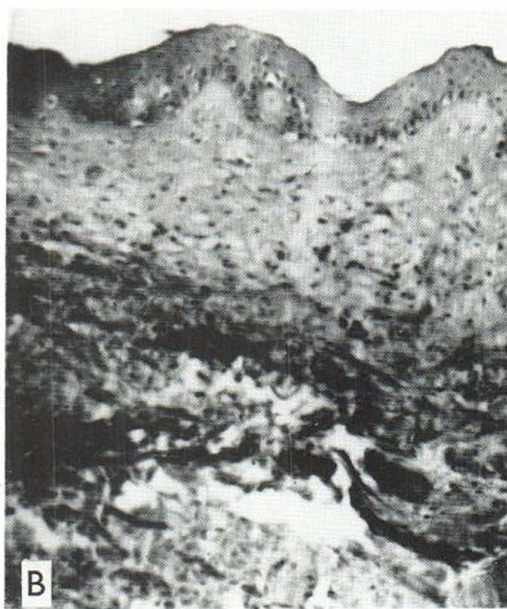
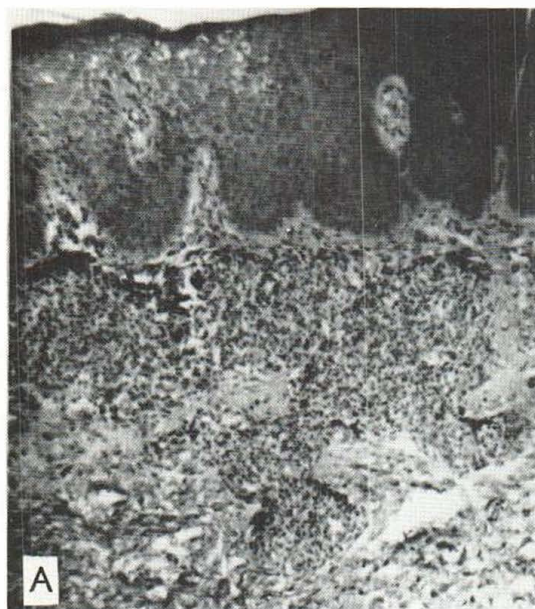


Fig. 1A and B. Mycosis fungoides: dermal infiltrate before and after PUVA.

tioned on a Reichert OM U2 ultramicrotome. The specimens were stained with uranyl acetate and lead citrate and examined with a Hitachi HU 12 A electron microscope.

RESULTS

Histology (Fig. 1a, b)

A reduction in the density of the dermal infiltrate was observed.

Electron microscopy in all biopsies performed after a total UVA dose of more than 30 J/cm².

Lichen planus

After photochemotherapy, a considerable reduction in the density of infiltrate was observed. The majority of lymphoid cells remaining in the superficial and mid-dermis showed pronounced changes. Three different appearances were observed: some lymphocytes possessed a rounded electron-dense nucleus, rich in heterochromatine and with an irregularly dilated perinuclear space revealing the nuclear pores. Cytoplasmic content was intact although the plasma membrane had partially disappeared (Fig. 2a). Other lymphocyte nuclei were isolated, very dense, without a nuclear membrane and sometimes partially disintegrated. Certain nuclei maintained a localized fringe of cytoplasm at one pole of the cell. This appearance was the most

frequently seen (Fig. 2b). Thirdly, nuclear remnants in the process of disintegration and phagocytosis by histiocytes were also observed (Fig. 3). In addition, many lymphocytes showing no nuclear or cytoplasmic alteration were present.

Mycosis fungoides

Before treatment, the infiltrate showed no alterations of proliferating cells (Fig. 4a) as in lichen planus. During and after treatment, a reduction in lymphocyte density with a high percentage of altered cells was observed. The changes observed were similar to those seen in lichen planus (Fig. 4b).

Table I. Biopsy specimens obtained from 6 patients

Figures within parentheses denote total J/cm² received at the time of biopsy. E, G, F, P=excellent, clinical results good, fair, poor

Patient	Before treatment	During treatment		
I (MF)	+	+ (43) F	+ (290) E	
II (LP)	+	+ (122) G		
III (MF)	+	+ (9) P	+ (26) F	+ (91) G
IV (MF)	+	+ (30) F		
V (MF)	+	+ (64) G		
VI (MF)	+	+ (46) F	+ (175) G	

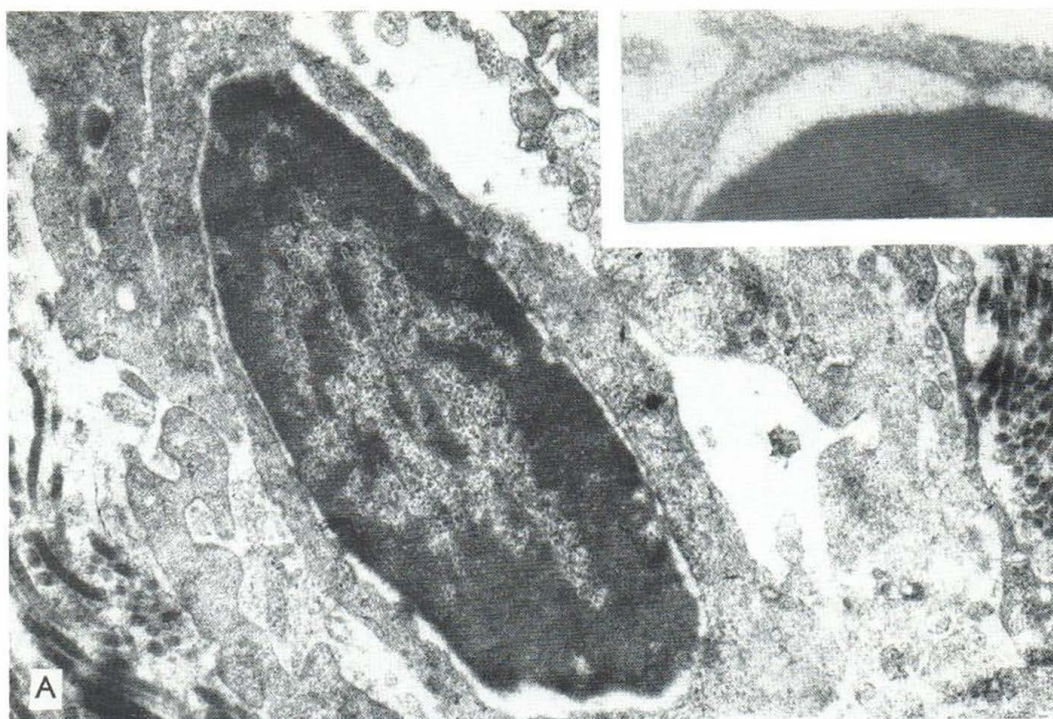


Fig. 2 A. Lichen planus: enlargement of the perinuclear space of a dermal lymphoid cell. $\times 22\,500$. Inset: $48\,600$.

Fig. 2 B. Lichen planus: isolated nucleus with narrow cytoplasmic fringe. $\times 18\,630$. Inset: localized absence of nuclear membrane, $\times 102\,060$.

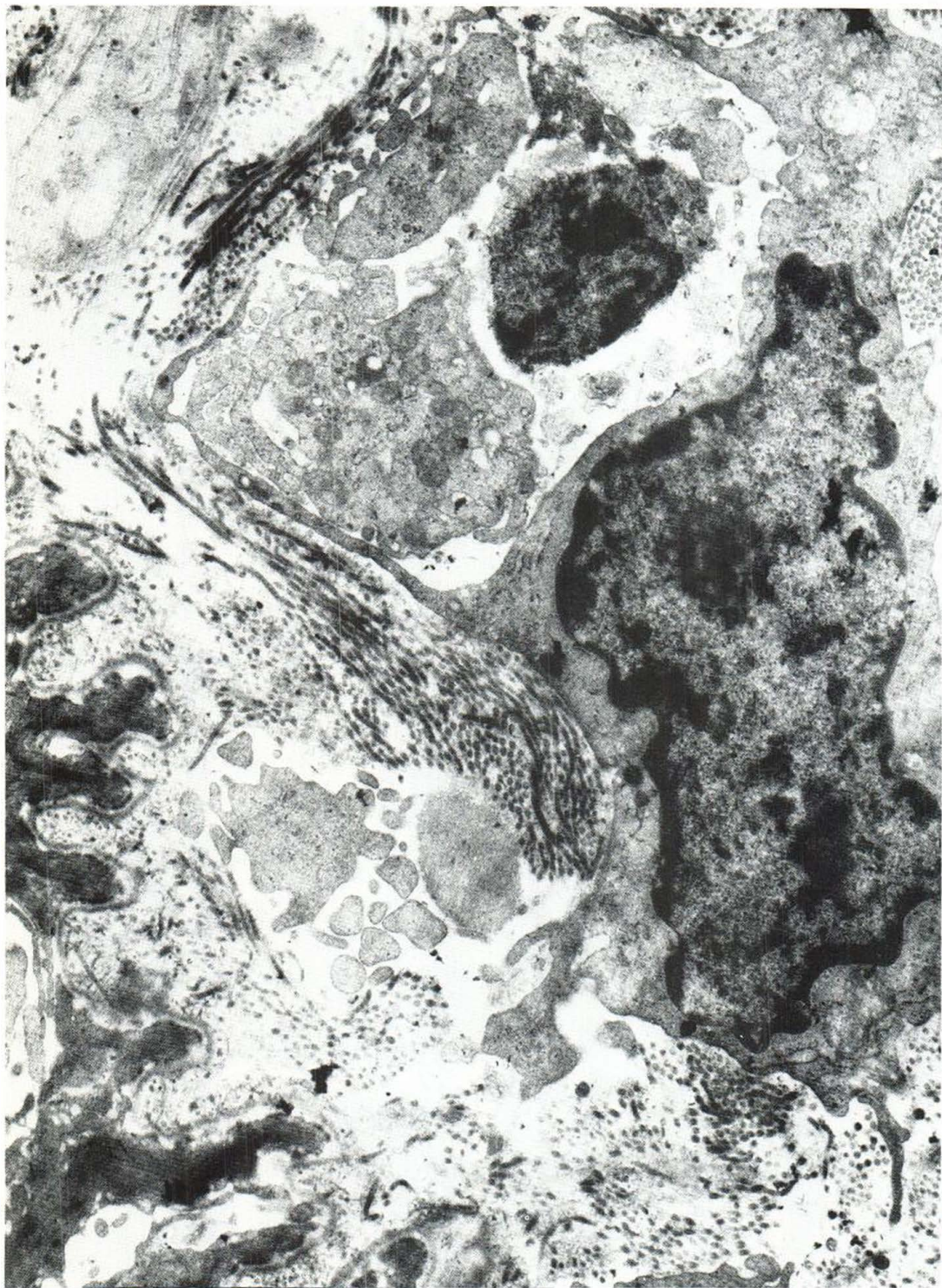


Fig. 3. Lichen planus: stage 3. Superficial dermis showing phagocytosis of cytoplasmic and nuclear debris. $\times 18\,360$.

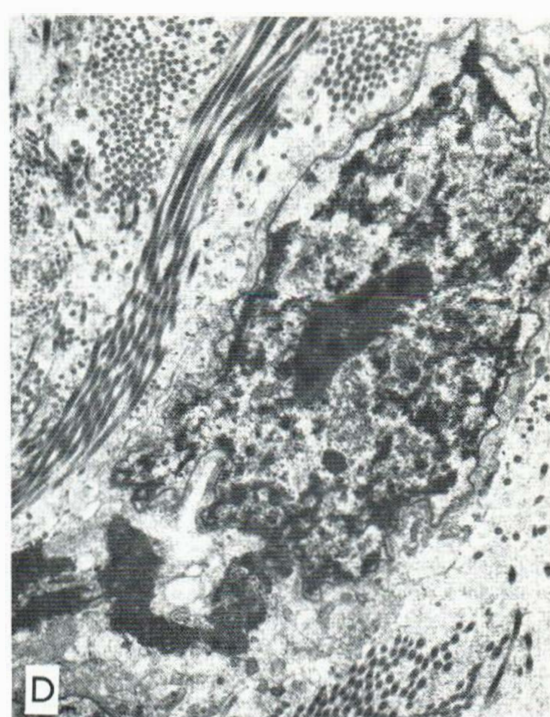
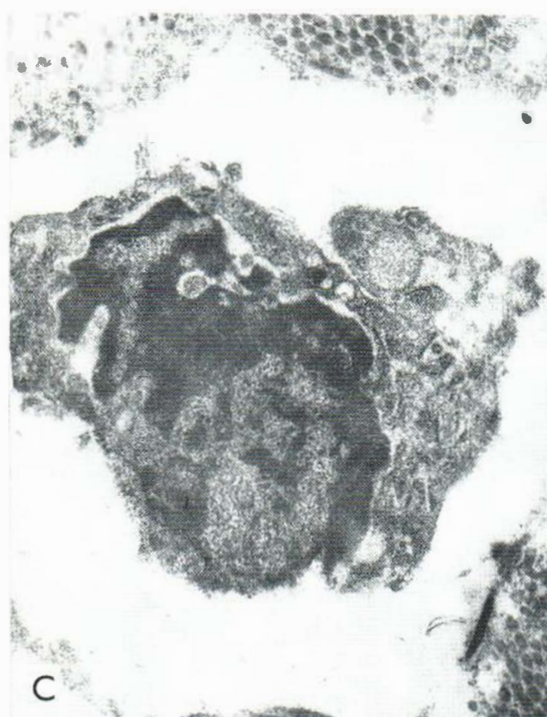
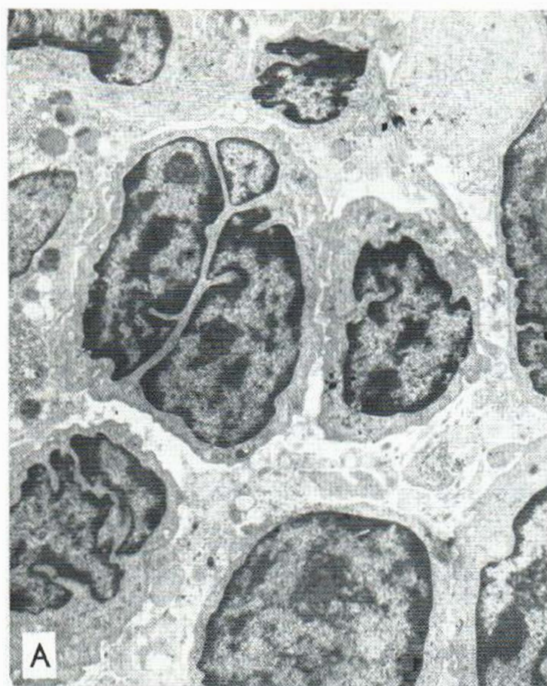


Fig. 4A. Mycosis fungoides: dermal infiltrate before PUVA. $\times 5865$.

Fig. 4B, C. Mycosis fungoides: dermal lymphoid cells of same patient during PUVA therapy. (B) $\times 25\,500$; (C) $\times 25\,500$.

Fig. 4D. Mycosis fungoides: lymphohistiocyte cell with abnormal nuclear heterochromatin scattered in small specks. $\times 11\,900$.



Fig. 5. Mycosis fungoides: appearance of vessels of superficial dermis in a case of MF during PUVA therapy. Deeply indented nuclei, abundant cytoplasmic filaments,

vacuolar separation of the intercellular space and duplication of the basal membrane. $\times 22\,540$.

4c). In addition, other changes were observed in some cells. These cells, larger than lymphocytes, contained an elongated nucleus with small indentations, scattered heterochromatin and small regular granules (mottes). The nucleolus was prominent and in the neighbourhood of these cells, isolated chromatin masses were seen (Fig. 4d).

In places, the endothelial cells of superficial blood vessels were also altered. The nucleus presented a convoluted appearance, cytoplasmic filaments were prominent, vacuoles were present. Below the cytoplasmic membrane there was an increase in villosity and considerable separation of the contact zones of epithelial cells. There was also marked basement membrane replication around the endothelium (Fig. 5).

DISCUSSION

The beneficial effect of oral photochemotherapy in certain dermatoses characterized histologically by a superficial dermal lymphocytic infiltrate is well recognized. Bot lichen planus (9) and mycosis fungoides (4, 5, 15) are improved by its therapeutic action. Histological examination of mycosis fungoides lesions after PUVA treatment shows disappearance of the cellular infiltrate from the superficial dermis to the level of the mid-dermis. This zone corresponds fairly accurately to the limit of penetration of UVA in the human dermis (1). Oral photochemotherapy probably has a direct effect on the proliferating lymphocytes in the dermal infiltrate of mycosis fungoides. The mechanism of disappearance has not been elucidated. Two hypotheses may be put forward—either arrest of proliferative capacity of the dermal lymphocytes, or frank destruction of the infiltrating cells. Our ultrastructural observations in 5 PUVA-treated patients with mycosis fungoides provide morphological evidence in favour of cellular destruction. The appearances of cell disintegration were found only during the course of treatment and were not found in pre-treatment biopsies. Approximately, 50% of the superficial dermal cells observed during treatment showed evidence of pre-necrosis or necrosis. Such changes were seen exclusively in the superficial dermis, which strongly suggests that photochemotherapy is the cause of the changes. Study of biopsies at various stages of treatment showed a progressive pattern of cellular change. Initially, the perinuclear space dilates without other evident nu-

clear or cytoplasmic alterations. The expansion of the perinuclear space then extends around the nucleus, ablating all nuclear cytoplasmic connections. This appearance of "cytoplasmic infarction" might represent the initial morphological event of the cellular damage induced by photochemotherapy in the dermal infiltrate of LP and MF, and constitutes evidence of cellular pre-necrosis.

Later, the cell cytoplasm becomes necrotic and disappears, leaving isolated lymphocyte nuclei with occasional preservation of cytoplasmic remnants. Eventually, nuclear necrosis, which has been proceeding in parallel, becomes complete. Cytoplasmic debris and pyknotic nuclear remnants are eliminated by macrophages. A similar sequence of events probably applies equally to the proliferating cells of MF and the more normal lymphocytes of LP. However, our experience of the changes in LP is limited, as only one case was examined.

Perinuclear vacuolar changes have been observed in psoriatic epidermis treated by oral photochemotherapy (14). This may be comparable to the appearance we have observed in superficial dermal lymphocytes in PUVA-treated MF and LP. However, we did not observe enormous vacuoles surrounding pyknotic nuclei, as was described by Vukas et al. (14). The notable heterochromatin alterations seen in some cells suggest a disturbance of nuclear metabolism, but precise interpretation is difficult.

Various recent investigative studies tend to confirm our interpretation of the morphological changes. Lymphocytes stimulated by PHA and treated with psoralens and ultraviolet light show evidence of degeneration (cytoplasmic vacuolization and nuclear pyknosis) which leads to cellular lysis (13).

Lymphocytes seem to be more disposed to the alterations induced by PUVA during the phase of DNA synthesis (12). Rapidly, proliferating lymphocytes may become more sensitive to the action of photochemotherapy than lymphocytes in a resting phase. T lymphocytes of the MF infiltrate probably have increased proliferative activity. It has been established that Sezary cells replicate in the skin more frequently in the epidermis than in the dermis (11). In lichen planus, numerous lymphoblasts have been found by autoradiography (8), proliferating cells probably constitute a population which is particularly susceptible to PUVA therapy.

However, the above studies show that the per-

centage of replicating cells in the skin is very low compared with the total density of the infiltrate. It is therefore unlikely that PUVA exerts its destructive influence on proliferating cells alone.

The changes seen in the endothelial cells of superficial dermal vessels, similar to those observed by Kumakiri, Hashimoto and Willis (7) cannot be easily correlated with the improvement of the lesions of MF or LP under PUVA treatment. These preliminary observations suggest that further experimental work is required concerning the effects of PUVA therapy on the skin and its cellular components.

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REFERENCES

1. Everett, M. A., Yeagers, E., Sayre, R. M. & Olson, R. L.: Penetration of epidermis by ultraviolet rays. *Photochem Photobiol* 5: 533, 1966.
2. Fitzpatrick, T. B.: Medical problems to enhanced ultraviolet in stratospheric ozone depletion. Hearing before the subcommittee on the upper atmosphere of the committee on aeronautical and space sciences. United States Senate. 99th Congress. Printed for the use of the Committee Aeronautical and Space Sciences, 464. US Government Printing Office, Washington, 1975.
3. Gilchrist, B. A., Parrish, J. A. & Tannenbaum, L.: Oral methoxsalen photochemotherapy of mycosis fungoides. *Cancer* 38: 683, 1976.
4. Hjortshøj, A. & Schmidt, H.: Photochemotherapy of mycosis fungoides. *Dan Med Bull* 24: 40, 1977.
5. Hodge, L., Vella Briffa, D., Warin, A. P., Gange, R. W. & Bleeher, S.: Photochemotherapy in mycosis fungoides. *Br Med J* i: 1257, 1977.
6. Kraemer, K. H. & Weinstein, G. D.: Decreased thymidine incorporation in circulating leukocytes after treatment of psoriasis with psoralen and long wave ultraviolet light. *J Invest Dermatol* 69: 211-214, 1977.
7. Kumakiri, M., Hashimoto, K. & Willis, I.: Biological changes due to long wave ultraviolet irradiation on human skin: ultrastructural study. *J Invest Dermatol* 69: 392-400, 1977.
8. Lachapelle, J. M. & De La Brassine, M.: The proliferation of cells in the dermal infiltrate of lichen planus lesions. *Br J Dermatol* 89: 137-141, 1973.
9. Ortonne, J. P., Thivolet, J. & Sannwald, C.: Oral photochemotherapy in the treatment of lichen planus (LP). Clinical results, histological and ultrastructural observations. *Br J Dermatol* 99: 1, 77-78, 1978.
10. Parrish, J. A., Fitzpatrick, T. B., Tannenbaum, L. & Pathak, M. A.: Photochemotherapy of psoriasis with oral methoxsalen and long wave ultraviolet light. *N Engl J Med* 291: 1207, 1974.
11. Saglier-Guedon, I., Prunieras, M., Durepaire, R. & Grupper, C.: Preferential replication of Sezary cells in the epidermis. *Bull Cancer* 64: 259-266, 1977.
12. Scherer, R., Kern, D. & Braun-Falco, O.: UVA induced inhibition of proliferation of PHA stimulated lymphocytes from humans treated with 8 methoxy-psoralen. *Br J Dermatol* 97: 519, 1977.
13. Schmoekel, C., Scherer, R., Kern, B. & Braun-Falco, O.: The cytolytic effect of PUVA treatment on PHA-stimulated human peripheral lymphocytes. *Acta Dermatovener (Stockholm)* 58: 203, 1978.
14. Vukas, A., Vefl, D. & Gligora, M.: Ultrastructural features of psoralen plus black light induced successive changes in psoriatic cell. *Dermatologica* 154: 277, 1977.
15. Roenigk, H. H., Jr: Photochemotherapy for mycosis fungoides. *Arch Dermatol* 113: 1047, 1977.

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J. Thivolet, M.D.
Clinique Dermatologique
Hôpital Édouard Herriot
F-69374 Lyon Cedex 2
France