

## THE CYTOLYTIC EFFECT OF PUVA TREATMENT ON PHA-STIMULATED HUMAN PERIPHERAL LYMPHOCYTES

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**Abstract.** The morphology of PUVA-treated and PHA-stimulated human peripheral lymphocytes *in vitro* was studied by means of the light and electron microscopes. Most cells showed signs of cytolysis, such as pyknotic nuclei and a swollen and decomposed cytoplasm; a few cells, however, appeared as normal lymphoblasts. In the latter, repair-enzyme mechanisms may successfully have rectified alterations in the DNS prior to the inception of S-phase. These findings are discussed with reference to the *in vivo* effect of PUVA treatment.

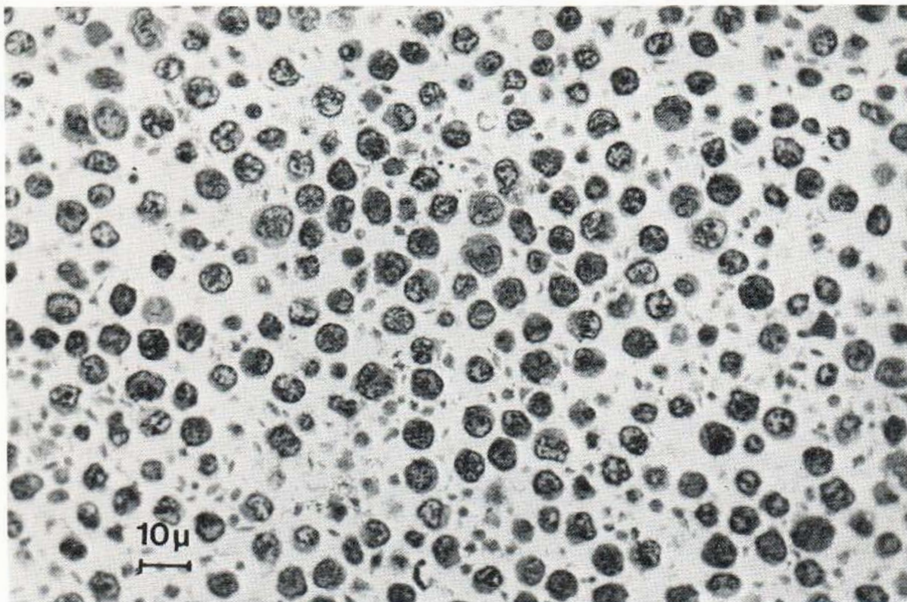
**Key words:** PUVA, psoralen; PHA-stimulated lymphocytes

Treatment with psoralen and longwave ultra-violet light (PUVA) has become a widely-used method in the past few years and its importance in and application to various diseases are still increasing (17). However, the mechanism of this type of photo-

therapy and the reason for its success when applied in unrelated diseases such as psoriasis (11, 28) and mycosis fungoides (12), particularly with regard to possible harmful side effects (23), need further clarification.

Previous reports have shown that the combination of psoralen and UVA (320–400 nm) inhibits DNA synthesis in various cell types such as keratinocytes (10, 26), human amnion cells (25), Ehrlich ascites tumour cells (3), and PHA-stimulated human peripheral lymphocytes (19, 20). It has also been demonstrated that this effect is attributable to the tendency of psoralens to cova-

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*Fig. 1.* Peripheral lymphocytes (semi-thin section) cultured over a period of 3 days.  $\times 680$ .

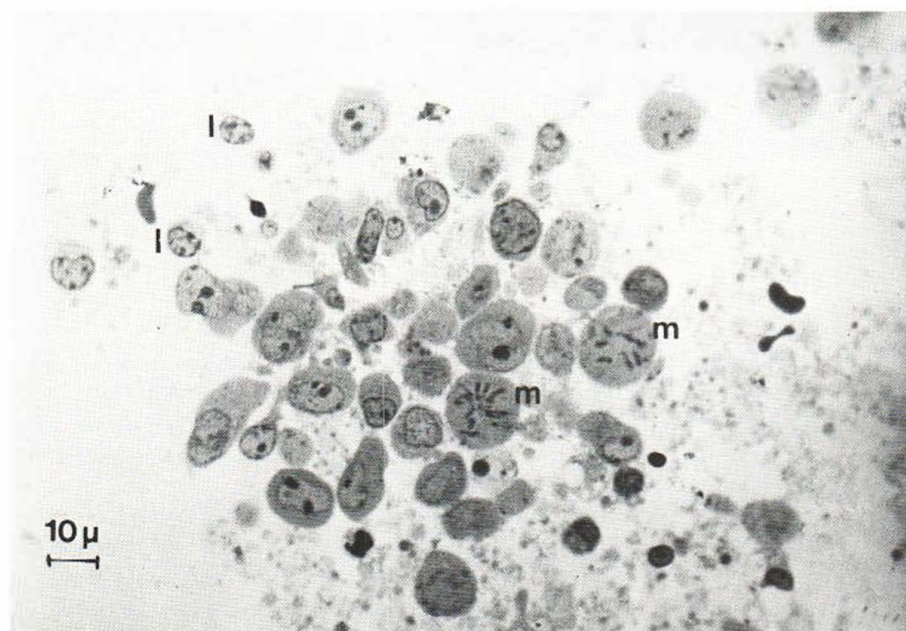


Fig. 2. PHA-stimulated lymphocytes (semi-thin section) showing lymphoblasts characterized by an abundant cytoplasm and by large euchromatic nuclei with distinct nucleoli. Two mitotic figures (*m*) are seen, as well as a few non-transformed lymphocytes (*l*).  $\times 680$ .

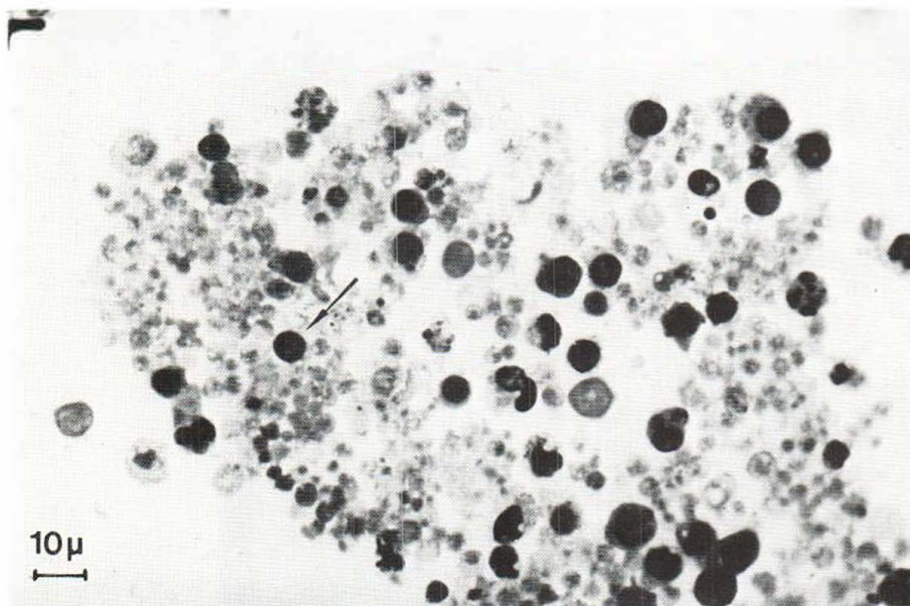


Fig. 3. PHA-stimulated and PUVA-treated lymphocytes (semi-thin section). Note the presence of highly pyknotic nuclei occasionally devoid of a surrounding cytoplasm ( $\rightarrow$ ).  $\times 680$ .

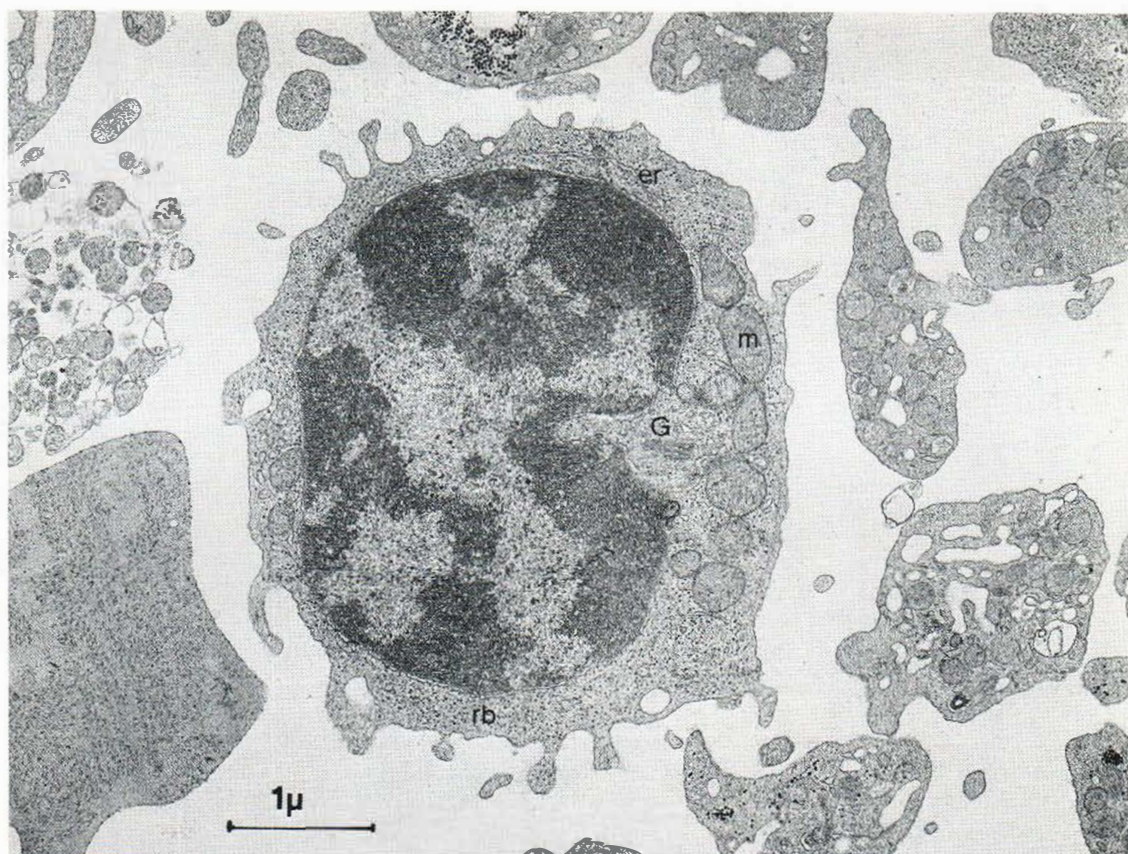


Fig. 4. Human peripheral lymphocyte (thin section) cultured in the absence of PHA over a period of 3 days. Note the presence of free ribosomes (*rb*), of mitochondria (*m*), of little endoplasmic reticulum (*er*), and of a Gol-

gi complex (*G*). The nucleus is mostly heterochromatic, with the nucleoplasm condensed along the nuclear membrane. There is no nucleolus.  $\times 19000$ .

lently bind to pyrimidine bases in DNA and to a lesser extent in RNA (18), thereby building inter-strand cross-links between opposing pairs of pyrimidine bases in the double-stranded DNA (7, 9).

Table 1. Summarized results of human peripheral lymphocyte cultures stimulated and non-stimulated with PHA and treated with UVA, 8-MOP, and PUVA

|        | Controls     | UVA          | 8-MOP        | PUVA                        |
|--------|--------------|--------------|--------------|-----------------------------|
| No PHA | Lymphocytes  | Lymphocytes  | Lymphocytes  | Lymphocytes                 |
| PHA    | Lymphoblasts | Lymphoblasts | Lymphoblasts | Cytolysis, few lymphoblasts |

Repair mechanisms of damaged DNA have been demonstrated (1, 8), and chromosomal aberrations (22) and sister chromatid exchanges (6) were interpreted as indications thereof. In this context, it is interesting to note that PUVA treatment has been shown to be lethal to bacteria (14, 16), to induce respiration-deficient mutants in yeast (21), to reduce DNA replication and cell division (2), to inactivate Ehrlich ascites tumour cells (15), and to cause damage (pyknosis, cytolysis) in some individual keratinocytes (27).

In order to gain further insight, we directed our attention to the fine structure of cells whose DNA synthesis is inhibited through PUVA treatment. Human peripheral lymphocytes, stimulated with phytohaemagglutinin (PHA) were used as an in vitro model system, as has had been proposed previously (19, 20).

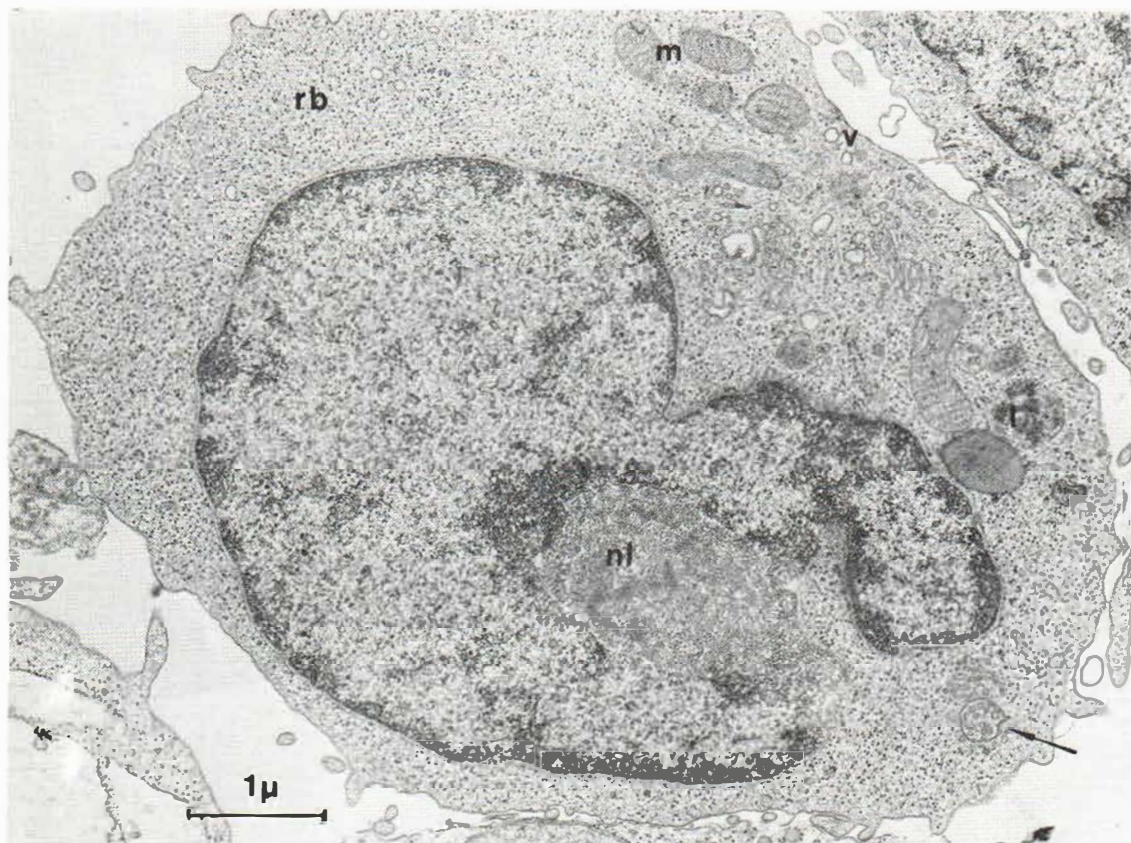


Fig. 5. PHA-stimulated lymphocyte (thin section). Its nucleus is mostly euchromatic, with only a little nucleoplasm condensed along the nuclear membrane and demonstrating large nucleolus (*nl*). In the cytoplasm, many

free ribosomes (*rb*), some mitochondria (*m*), vesicles (*v*), lysosomes (*l*), and a multivesicular body (→) are noted.  $\times 18\,000$ .

## MATERIAL AND METHODS

1. *Peripheral human lymphocytes* were isolated and prepared as described previously (19). 2 ml of each cell preparation was placed in a 15 ml plastic test tube and 8 ml medium TCM 199 (Gibco Bio-Cult, Glasgow, Scotland) together with 0.8 ml autologous serum was added.

2. *8-Methoxypsoralen (8-MOP)* was taken from Oxsoralen® capsules (Paul B. Elder Co., Byron, Ohio). Two concentrations were used: (a) 1.1  $\mu\text{g/ml}$  (the concentration was prepared by serial dilution in culture medium; no alcohol was used). (b) in vivo concentration (achieved by taking lymphocytes from donors 2 hours after the oral intake of 8-MOP at the dosage currently used for psoriatic patients (20).

3. *UVA irradiation.* The lymphocyte preparation was contained in test tubes sealed with adhesive tape. These were irradiated in the same black light unit used in our clinic for treatment of patients (UVA-lying box, type SIV, equipped with 70 Sylvania GTE fluorescent lamps,

manufacturer Seliger GmbH, Vienna). The UVA output was 4.5  $\text{mW/cm}^2$ . The irradiations lasted for 15 and 30 minutes, corresponding to average doses currently used for patients, viz. 4 and 8  $\text{Joule/cm}^2$ .

4. *Lymphocyte transformation.* 0.1 ml per  $2\text{--}5 \times 10^6$  cell-s/ml of reconstituted phytohaemagglutinin HA 15 (Wellcome Research Laboratories, Beckenham, England) was added immediately. Cultures were then incubated for 3 days at 37°C.

5. The cultures were centrifuged at 500 *g* for 10 minutes. The lymphocytes were then fixed in 2% paraformaldehyde and in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) for 1.5 hours at 4°C and rinsed twice in the same medium. The final fixation consisted of 1%  $\text{OsO}_4$  (dissolved in medium TCM 199) for 1 hour at 4°C. The cellular agglomerations were then dehydrated and embedded in Epon.

6. The embedded material was cut on a Reichert OmU2 ultramicrotome. Semi-thin sections were stained with methylviolet and evaluated with a light microscope. Thin sections were post-stained with uranyl acetate and lead

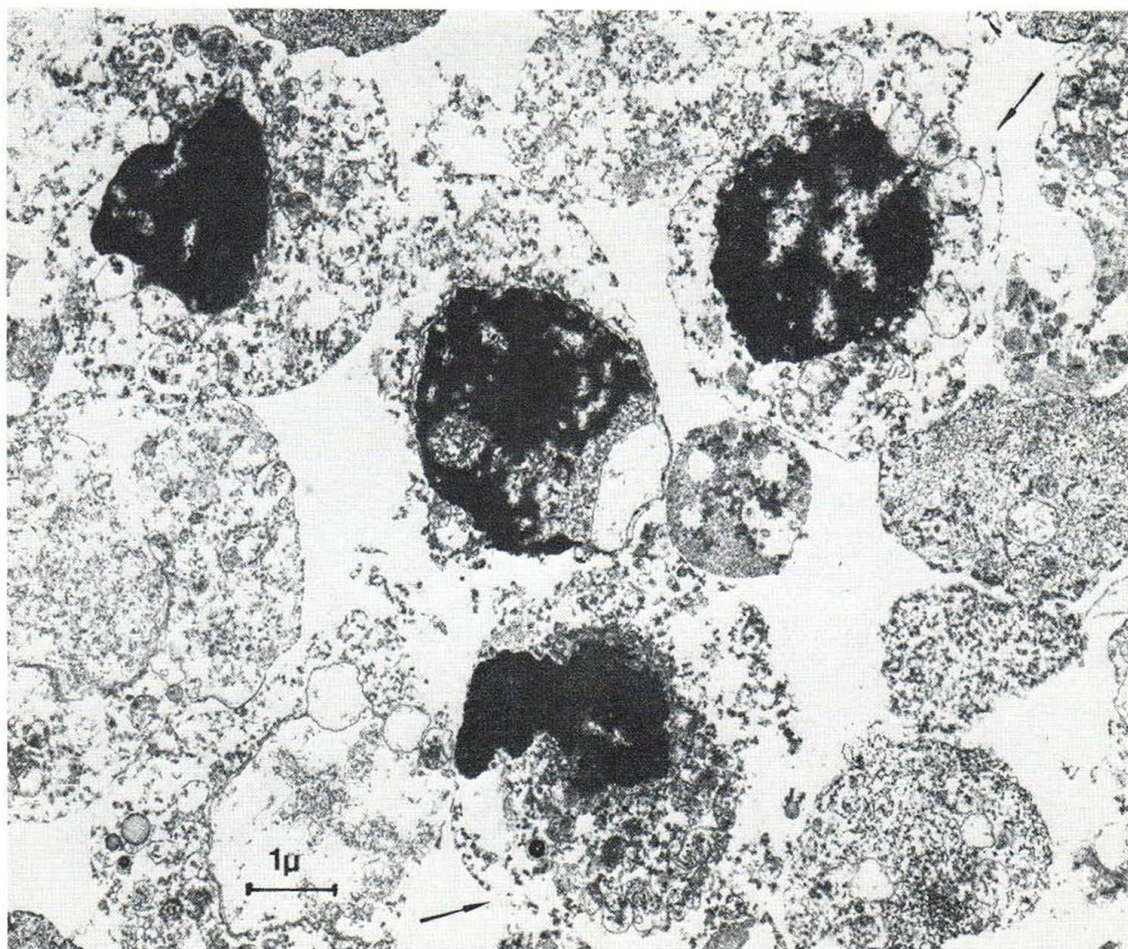


Fig. 6. PHA-stimulated and PUVA-treated lymphocytes (thin section) showing lethally damaged cells. The nuclei are small and pyknotic and the cytoplasm is swollen and

decomposed. The cytoplasmic and nuclear membranes often appear to disintegrate ( $\rightarrow$ ).  $\times 11\,300$ .

citrate and studied with a Philips EM 300 electron microscope.

7. PUVA-treated and PHA-stimulated human peripheral lymphocytes and controls were also studied in the scanning electron microscope (Cambridge Mark IIa, beam voltage 27 kV) after being prepared by critical-point dehydration.

8. Controls were carried out omitting either 8-MOP or UVA irradiation or both, and either with or without PHA stimulation. (For further details, see Scherer et al., 1977.)

## RESULTS

### A. Semi-thin sections

1. Controls without PHA showed normal lymphocytes characterized by their small size, sparse cytoplasm and dense nuclei without nucleoli (Fig. 1).

Also present were a few monocytes and erythrocytes. No difference was noted between lymphocytes treated with PUVA, with 8-MOP alone, with UVA alone, or not treated with any of these (Table I).

2. Controls with PHA added showed lymphoblasts characterized by large lymphoid cells exhibiting an abundant cytoplasm and large euchromatic nuclei with distinct nucleoli (Fig. 2). Also present were mitotic figures, a few normal-appearing lymphocytes and occasionally necrobiotic cells. Controls treated with UVA or with 8-MOP alone showed no differences (Table I).

3. PHA-stimulated and PUVA-treated lymphocytes showed mostly cells with pyknotic nuclei and very clear cytoplasm (Fig. 3). Some lymphoblasts

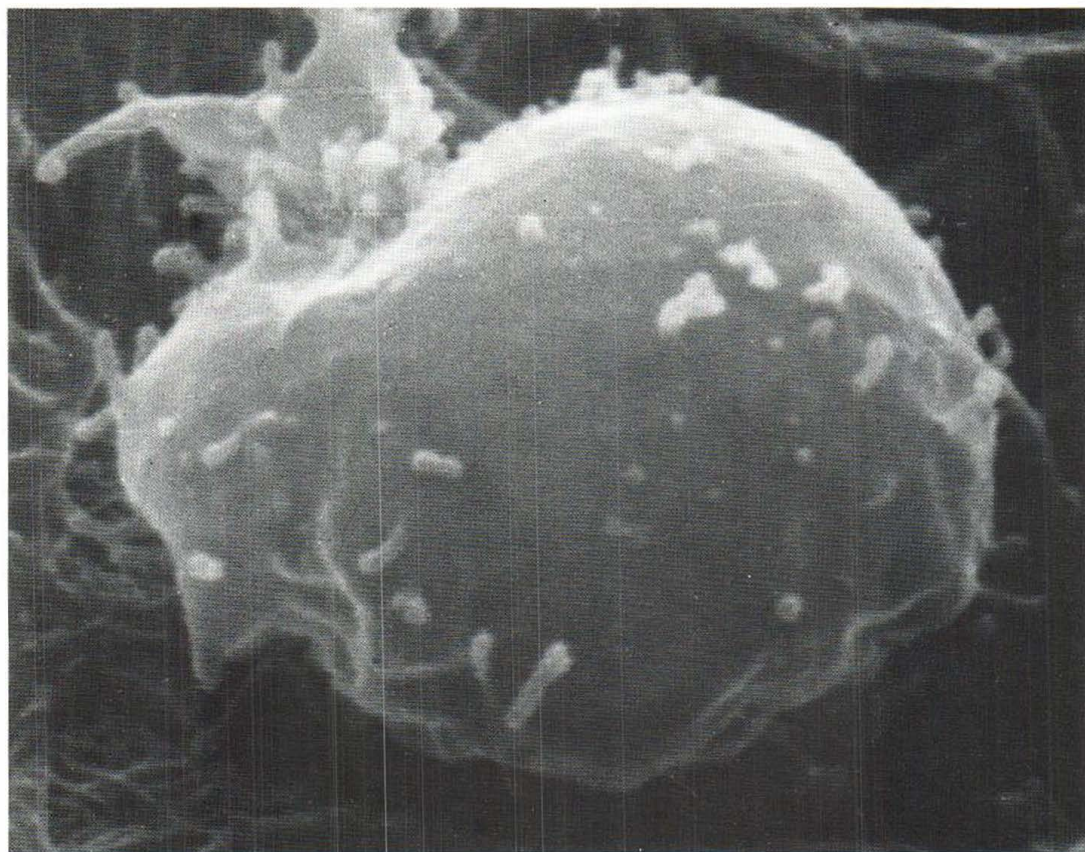


Fig. 7. Human T-lymphocyte (scanning electron micrograph) prior to stimulation with PHA.  $\times 18\,900$ .

and non-stimulated lymphocytes were observed, however, and these were more numerous in cultures irradiated with UVA for only 15 minutes. No difference was noted between the *in vitro* concentration ( $1.1\ \mu\text{g/ml}$ ) and the *in vivo* concentration (Table I).

#### *B. Thin sections*

1. Controls without addition of PHA showed normal lymphocytes: their nuclei were small and the chromatin was condensed along the nuclear membranes. The cytoplasm was sparse and contained few mitochondria and free ribosomes, as well as very little endoplasmic reticulum (Fig. 4).

2. Controls with addition of PHA showed lymphoblasts. Their nuclei were large and contained well-developed nucleoli and little chromatin-condensation along the nuclear membranes. The cytoplasm was abundant and exhibited many

free ribosomes, some mitochondria, but little endoplasmic reticulum (Fig. 5). No alterations were seen in lymphoblasts treated with 8-MOP or with UVA alone.

3. PHA-stimulated and PUVA-treated lymphocytes showed many necrobiotic cells whose nuclei were small and pyknotic and whose cytoplasm was swollen and completely decomposed. The cytoplasmic and nuclear membranes often appeared to disintegrate (Fig. 6). Some lymphoblasts and non-stimulated lymphocytes present showed no ultrastructural alterations.

#### *C. Scanning electron microscopy*

No significant surface alterations or lesions could be seen by scanning electron microscopy in lymphocytes fixed and prepared immediately after PUVA treatment, as compared with untreated controls (Fig. 7). After 3 days of lymphocyte cell

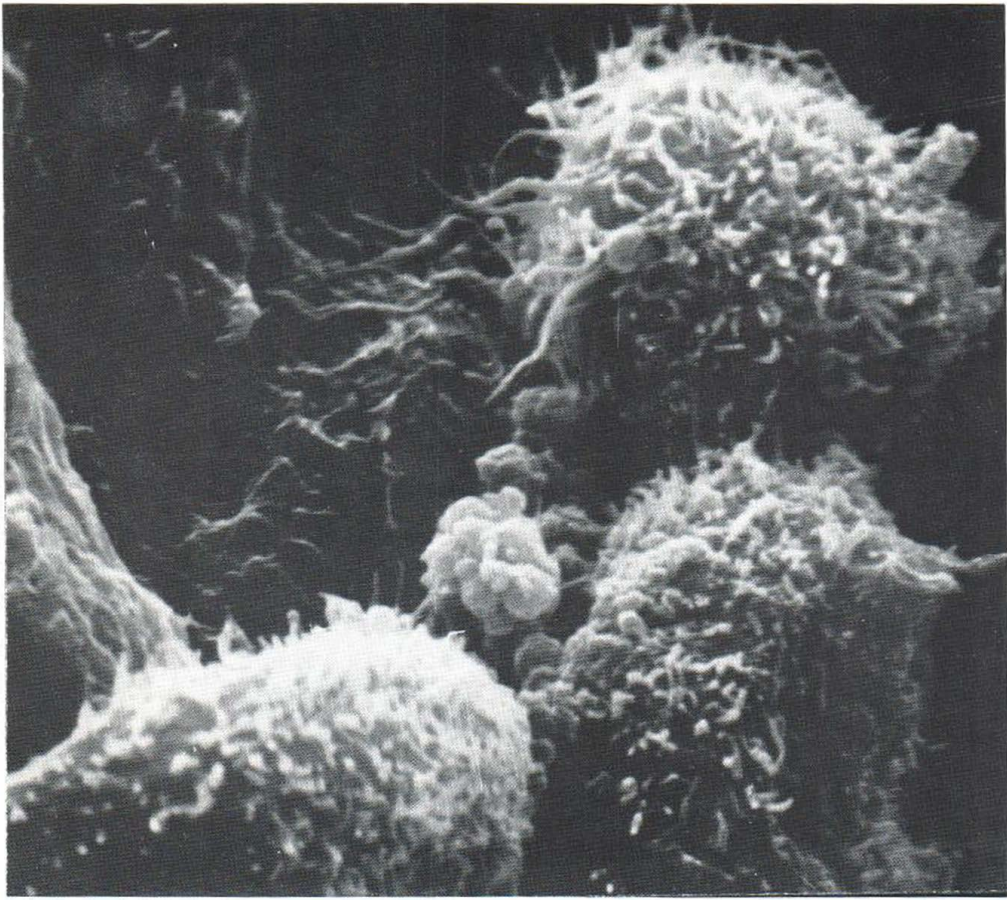


Fig. 8. PHA-induced lymphoblast transformation in the absence of PUVA treatment as viewed in the scanning electron microscope.  $\times 3\,200$ .

culture in the presence of PHA, the control cultures showed normal blast transformation with cellular enlargement and development of a typical villous surface architecture (Fig. 8). By contrast, lymphocytes from PUVA-treated cultures rarely showed blast transformation. Many of these lymphocytes seemed to have numerous membrane lesions scattered over the cell surface (Fig. 9).

#### DISCUSSION

Our results indicate that the combined effect of psoralen and UVA results in the death of cells entering the cell cycle ( $G_1$ - to S-phase). Resting cells ( $G_0$ -phase) are not visibly affected, possibly because DNA damage, if it occurs, is compensated, probably via repair-enzyme mechanisms (1, 6, 8).

Apparently these repair mechanisms cannot operate properly when the total DNA is activated. Transcriptional processes may no longer be possible, leading to a reduction of RNA and protein synthesis and to the inhibition of vital cellular functions. However, whether repair-enzyme mechanisms themselves (1) and RNA (18) and protein synthesis are inhibited in stimulated lymphocytes needs further clarification.

The presence of a few lymphoblasts in PUVA-treated and PHA-stimulated lymphocyte cultures may serve to indicate the significance of these repair mechanisms. It is known that the lymphocytes are not transformed simultaneously; a few start DNA synthesis as early as 1 hour after addition of PHA, whereas others only after 48 hours or more (24). It is conceivable that some PUVA-treated

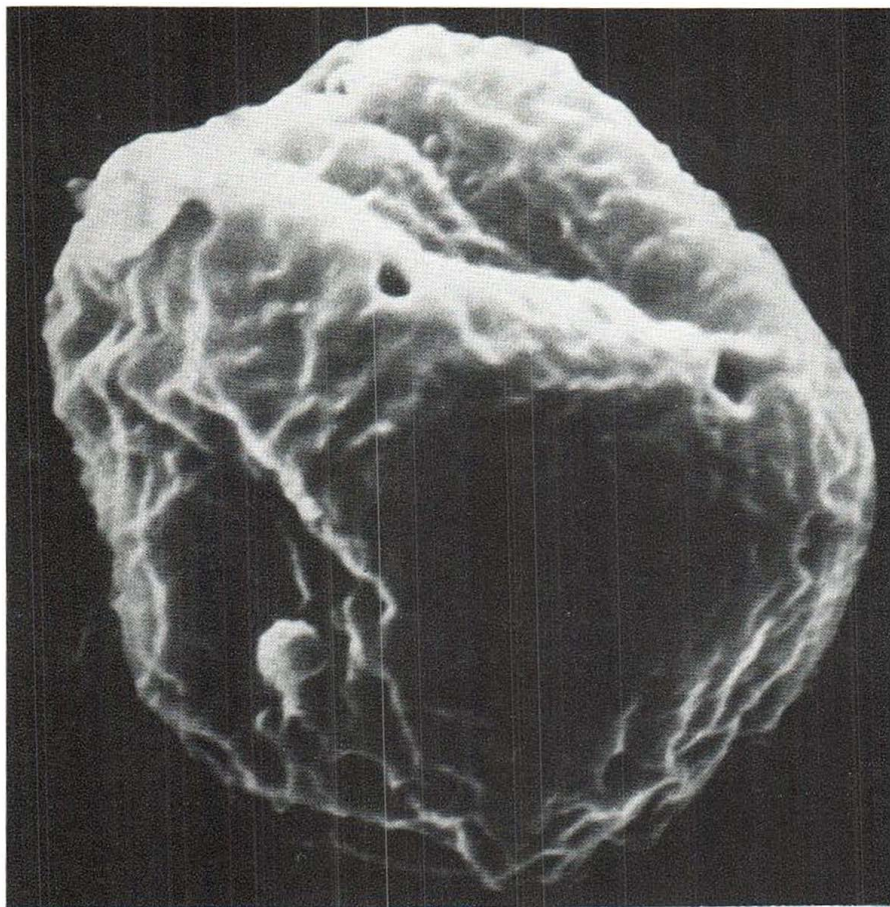


Fig. 9. Lymphocyte (scanning electron micrograph) from a PUVA-treated 3-day lymphocyte culture stimulated with

PHA. The lymphocyte cell membrane shows a number of hole-like lesions.  $\times 20\,700$ .

lymphocytes had successfully repaired DNA damage before commencing transformation into lymphoblasts.

Apparently the effect of PUVA treatment does not depend on the type of cell, since the inhibition of DNA synthesis has been shown for various cells (3, 10, 19, 25, 26). This explains the beneficial effect in different diseases: in mycosis fungoides, proliferating neoplastic lymphocytes (5) are most probably affected. In psoriasis, the inhibition of proliferating keratinocytes seems to be more important than the inhibition of cells in the dermal infiltrate, as DNA synthesis has been shown to increase insignificantly in the dermal cellular infiltrate of psoriasis (13) unless the psoriasis is of the erythroderma variant (4).

The question arises whether the proliferating cells *in vivo* are lethally damaged in the same way as they were in our *in vitro* experiments. Ultrastructural investigations of human skin treated with UVA and 8-MOP (topically applied) revealed intracellular edema and occasional cytolysis of individual keratinocytes. These alterations were less pronounced when 8-MOP was taken systemically and no cytolysis was observed (27). It is possible that the individual cells for which these findings have been described were the ones entering the cell cycle at the time of the irradiation. However, whether cytolysis represents a characteristic *in vivo* effect of PUVA treatment remains doubtful and must be clarified through further investigations.

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