Ultrastructural Modification of the Plasma Membrane in HUT 102 Lymphoblasts by Long-Wave Ultraviolet Light, Psoralen, and PUVA

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Ultrastructural alterations of the plasma membrane in HUT 102 lymphoblasts were assessed after a 2-h interaction with a suprapharmacologic (15 μg/ml) concentration of 8-MOP, 2-h irradiation with UVA (2.1 mW/cm²), and the exposure of the HUT 102 cells to PUVA under the same conditions.

The dark reaction of HUT cells with 8-MOP resulted in the disappearance of microvilli, the emergence of plasma-membrane–associated spherical bodies, formation of lamellar fungiform membrane evaginations, and, in approximately 1% of the cells, formation of uropods and cell capping. Except for uropod formation and cell capping, UVA has induced the same plasma-membrane alterations, and was more deleterious to structural cytoplasmic integrity than 8-MOP. Morphologic changes of the plasma membrane in PUVA-exposed cells tended to replicate structural alterations elicited independently during the dark reaction by suprapharmacologic 8-MOP concentrations. Partial retention of microvilli by cells after PUVA was the sole exception.

In light of all available evidence we conclude that psoralen during the dark reactions interacts with plasma membrane lipids by as yet undisclosed mechanisms and that in addition to lipids, membrane proteins are also the primary target of the initial interaction of HUT 102 cells with psoralen during PUVA treatment. *J Invest Dermatol 95:97–103, 1990*

In the presence of exogenous photosensitizers, photomodification of biologic systems by long-wave ultraviolet light is determined by UVA irradiation fluencies, chemical properties of a sensitizer in the absence of light, its reactivity when photoactivated, and the nature and response of the specific target to the photosensitization process. It follows therefore that photosensitization may be viewed as temporal superposition by a number of independent but concurrent reactions, ultimately expressed as a specific bioeffect.

For instance, the initial interaction of psoralen, a widely employed furocoumarin photosensitizer, with a number of eukaryotic cells is now known to proceed via binding of this ligand by specific, low- and high-affinity plasma-membrane psoralen receptors [1]. Thereafter, activation of membrane-bound psoralen results in covalent modification of the binding sites [1], and implicitly in structural membrane photomodification. Subsequently, the inhibition of epidermal growth factor (EGF) binding [2] and EGF receptor expression [3] by photosensitized cells was demonstrated.

We have shown previously that the structure of erythrocyte ghosts is altered by the dark reaction with psoralen, and that photomodification of ghosts by PUVA results in the formation of ghost-derived multilamellar bodies [4]. Similarly, a transient enhancement of plasma-membrane permeability to 5-iodo-2′-deoxyuridine (IdUrd) during the dark reaction of HUT 102 lymphoblasts with suprapharmacologic psoralen concentration was demonstrated recently [5].

Our results and the evidence summarized elsewhere [6] jointly suggest that cell membranes very probably are the primary target of the initial interaction with furocoumarins and that functional photomodification of plasma membranes by psoralen must be accompanied by the corresponding structural membrane alterations.

In accordance with the premise we have evaluated the state of the plasma membrane and the ultrastructure of malignant HUT 102 lymphoblasts [7,8] in response to UVA irradiation, suprapharmacologic 8-MOP concentration, and PUVA.

MATERIALS AND METHODS

Materials The RPMI-1640 medium, Dulbecco's Ca^{++} and Mg^{++}-free phosphate-buffered saline (PBS) as well as penicillin (1585 units/mg) were obtained from Grand Island Biological Co. (GIBCO), Grand Island, NY. Streptomycin sulfate and sodium bicarbonate were from Eli Lilly and Co., Indianapolis, IN and from J.T. Backer Chemical Co., Phillipsburg, NJ. Fetal calf serum from Flow Laboratories (McLean, VA) was heat-inactivated for 30 min at 56°C. Bovine serum albumin was purchased from Sigma Chemical Co. (St. Louis, MO) and 8-MOP was from Paul B. Elder, Co., Bryan, OH. Fisher Scientific Co., Silver Springs, MD supplied trypan blue. Glutaraldehyde, sodium cacodylate, osmium tetroxide, and British Araldite were purchased from Polysciences, Inc., Warrington, PA. Uranyl acetate and lead citrate were obtained from Electron Microscopy Sciences, Fort Washington, PA. Mallinkrodt, St. Louis, MO, supplied tannic acid.

Methods Cultures of HUT 102 lymphoblasts [7,8] were grown, in RPMI-1640 medium supplemented with 10% v/v Hi-FCS, 100

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Abbreviations:
8-MOP: 8-methoxypsoralen
EGF: epidermal growth factor
Hi-FCS: heat-inactivated fetal calf serum
HTLV-1: human T-cell leukemia/lymphoma virus
IdUrd: 5-iodo-2′-deoxyuridine
PBS: phosphate-buffered saline
PUVA: 8-MOP and UV-A
UVA: long-wave ultraviolet light

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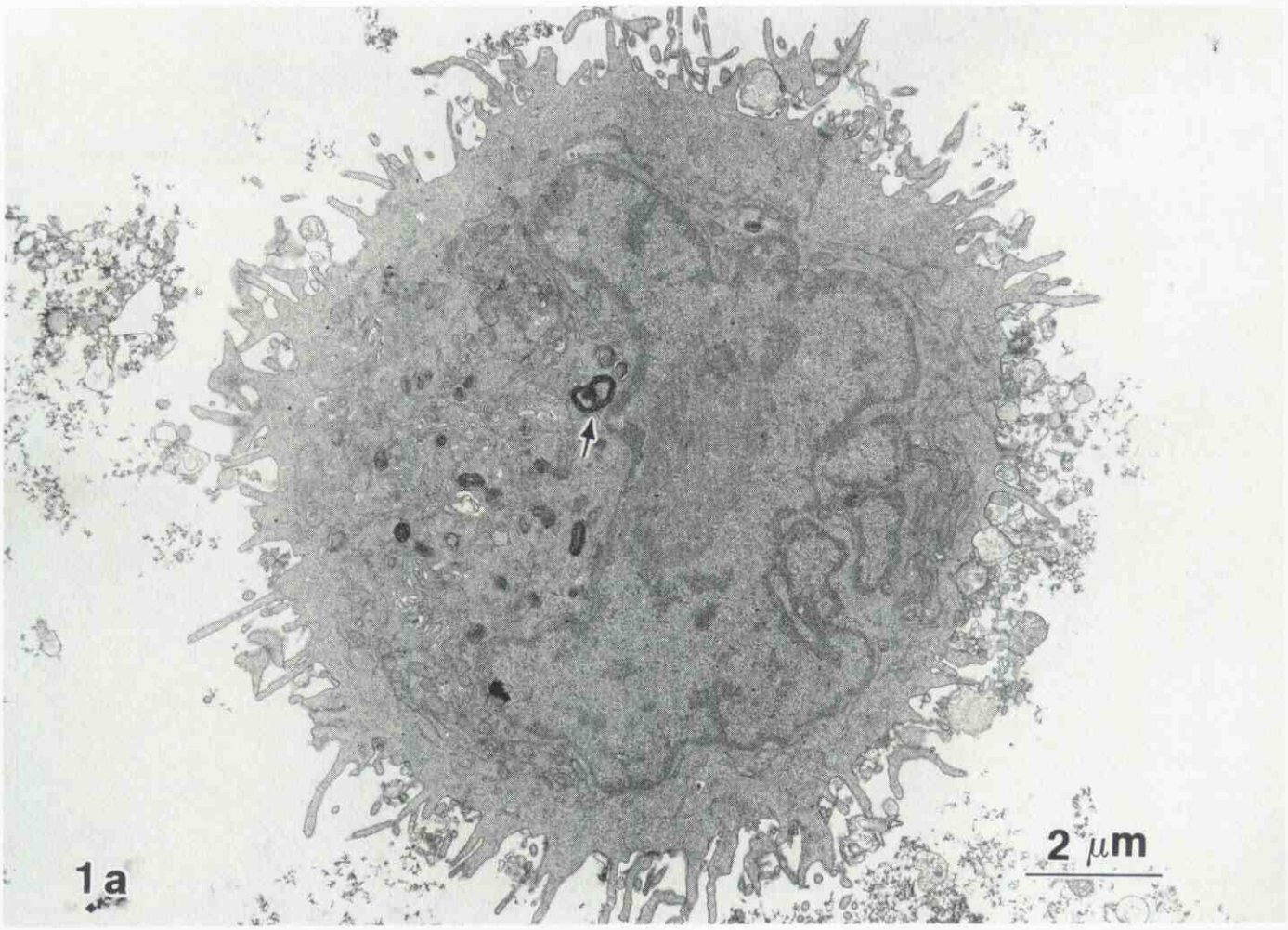


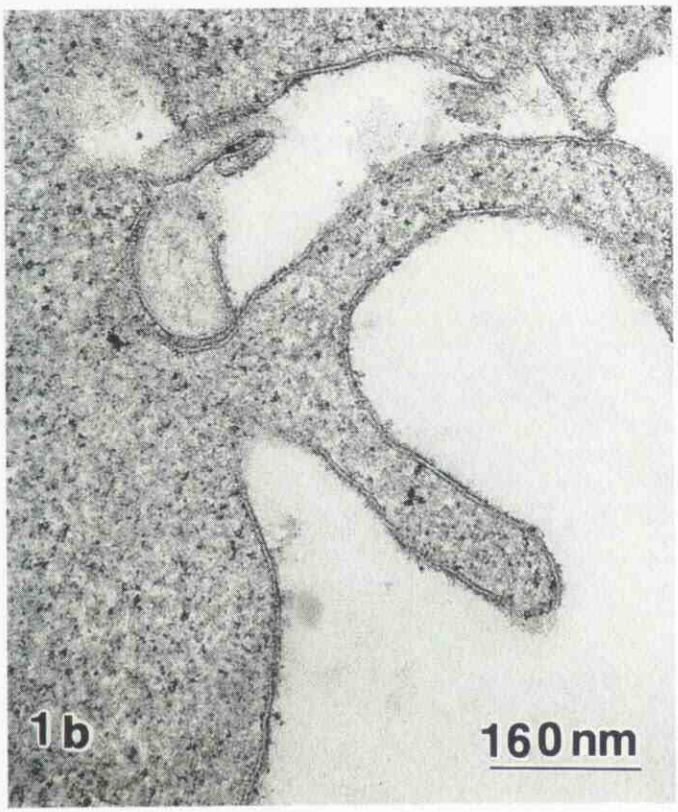


Figure 1. An intact HUT 102 lymphoblast. *a*: The plasma membrane of lymphoblast was characterized by a profusion of pleomorphic microvilli and concentric membrane-associated vesicles. The nuclei showed indentation with slight chromatin margination. Cytoplasm contained rough and smooth endoplasmic reticulum, lipid droplets, mitochondria, lysosomes and Golgi apparatus. Magnification ×9900. Note *(arrow)* the presence of lamellated intracytoplasmic body. *b*: Tannophilic plasma membrane of intact cells clearly showing its unit membrane structure. Inhomogeneity of membrane-adjacent cytoplasmic granules is also in evidence. Magnification ×126,000.

U/ml penicillin, 100 μg/ml streptomycin, and 0.9 mg/ml $NaHCO_3$. Exponentially growing cells were harvested, washed in three changes of PBS and then divided into four 1-ml aliquots containing 5×10^7 cells/ml each [5].

The first, control, aliquot was dispensed into a 5-ml pyrex beaker and agitated for 2 h on a rotating (60 rpm) platform at 25°C. The second aliquot of HUT 102 cells was incubated in the presence of 15 μg/ml 8-MOP under identical conditions. The third and the fourth aliquots were irradiated for 2 h with UV-A in the absence and presence of 15 μg/ml of 8-MOP. The irradiation of the third and fourth cell aliquots was performed as before [5] with an Ultraviolet Products Model XX-15 lamp emitting mainly at 365 nm with actinic intensity of 2.1 mW/cm² at 14 cm distance. During incubation of cells with 8-MOP and irradiation with UV-A, the volume of aliquots was maintained at a constant level by periodic additions of distilled water.

Viability of cultured cells was monitored before and after experimental procedures by trypan blue exclusion, and was found to fall exactly within the previously determined range [5].

Electron Microscopy Immediately upon termination of the concurrent procedures, all cells were washed with RPMI-1640 medium and then fixed for 2 h at ambient temperature with 2.5% v/v glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4). For the subsequent enhancement of cell-membrane staining the fixative was supplemented with 1% w/v tannic acid [9]. Fixed cells were pelleted by centrifugation and the cell pellets were washed with and resuspended in fresh 0.1 M cacodylate buffer (pH 7.4). Thereafter cells were post-fixed for 2 h with 1% w/v osmium tetroxide also dissolved in 0.1 M cacodylate buffer. Osmified cells were then dehydrated with graded ethanol solutions and embedded in British Araldite.

Ultrathin sections were cut with a diamond knife on LKB ultratome III and stained with uranyl acetate and lead citrate [10].

Stained sections were examined with a Philips 300 electron microscope.

RESULTS

Ultrastructure of Intact HUT 102 Lymphoblasts Plasma membrane of near diploid and hyperdiploid [5] HUT 102 cells was characterized by a profusion of pleiomorphic, slender microvilli and by the presence of concentric, membrane-associated vesicles (Fig 1*a,b*). Microvilli and cytoplasm were circumscribed by tannophilic plasma membrane exhibiting a clearly delineated unit membrane structure (Fig 1*b*). No lamellar membrane evaginations were noted in control cells. The hyaloplasm of intact cells was inhomogeneously granular and on occasion contained vacuoles and lipid inclusions. In some cells, morphologically characteristic HTLV-1 virus particles were detected. Strongly osmophilic, small electron dense bodies consisting of closely packed lamellae, numerous mitochondria, rough endoplasmic reticulum, and associated ribosome as well as Golgi network were well represented in most cells. Cell nuclei were frequently eccentric and lobulated and contained from one to several nucleoli. No striking morphologic differences were established between the nuclei of diploid and near diploid cells comprising HUT 102 cell cultures [5].

Effect of UV-A Irradiation on the Ultrastructure of HUT 102 Cells The most striking effect of 2-h UVA irradiation was inflicted on the plasma membrane of target cells. As a rule, the irradiation resulted in retraction of microvilli and their concomitant transformation into tapered, vallate cytoplasmic protrusions, thus imparting to cross-sections of irradiated cells a characteristically serrated form. The changes in plasma-membrane topology were accompanied by the almost complete disappearance of membrane-associated vesicles that, as was noted, profusely decorated the plasma membrane of intact cells. Besides the already-noted cell-membrane alterations, the irradiated cells were frequently decorated with the fungiform membrane evaginations and with plasma-membrane–associated extracellular round bodies. These extracellular bodies, 600 nm in diameter, appeared to consist of the densely packed concentric lamellae, which in their turn were encapsulated within one to several layers of the electron-opaque tannophilic envelope. The attended fungiform membrane evaginations or protrusions were firmly anchored by their stalk to the cell membrane, and for this reason were also continuous with the cytoplasm of the host cells. Structurally, these membranous evaginations consisted of the overlapping, multi-concentric lamellae forming an intricately connected network. Apart from the already-mentioned membranous evaginations, the remainder of the plasma membrane of irradiated cells remained essentially intact. Beside these morphologic changes, the cytoplasm of irradiated cells contained an increased number of lipid inclusions and vacuoles of different sizes. In a number of cells, the vacuoles and the attendant cytoplasmic lesions tended to encompass an extensive area (Fig 2). In photodamaged cells the intracytoplasmic lamellar bodies of increased dimensions were frequently present either on the periphery or within the confines of vacuolated cytoplasmic agglomerations. They markedly exceeded in size the analogous intracytoplasmic lamellar inclusions seen in intact cells. Other cell organelles of irradiated cells had an increased binding affinity for the electron-opaque cations and the mitochondrial cristae tended to become indistinct. The nuclear envelopes of irradiated cells likewise had an enhanced affinity for electron opaque stain and also displayed an accumulation of heterochromatin along the nuclear envelope. The irradiated cells failed to form uropods and no polarization of the cytoplasm or its components was observed.

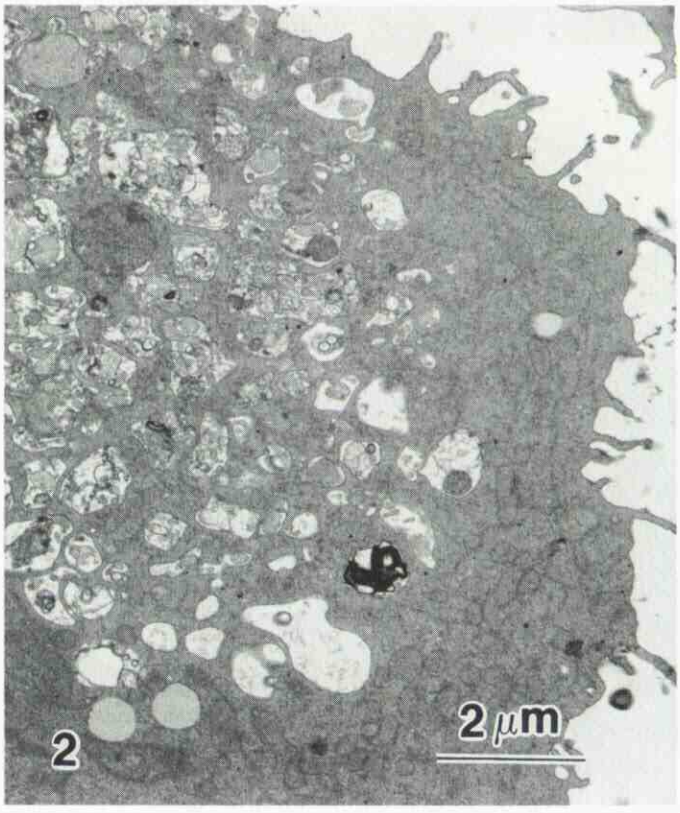


Figure 2. UVA-irradiated HUT 102 cells. In this case, the vacuoles and the attendant cytoplasmic lesions tended to encompass an extensive area. Magnification ×9900.

Effect of the Dark Interaction of 8-MOP with HUT 102 Cells on Their Ultrastructure Dark interaction of 8-MOP with HUT 102 cells resulted in the numerical reduction and retraction of microvilli (Fig 3). In addition, these changes in some cells were also accompanied by the appearance of perimembranous sinuses (Fig 3) possibly formed by fusion of the adjacent microvilli. Tannophilic and extracellular membrane-associated bodies, membrane-derived

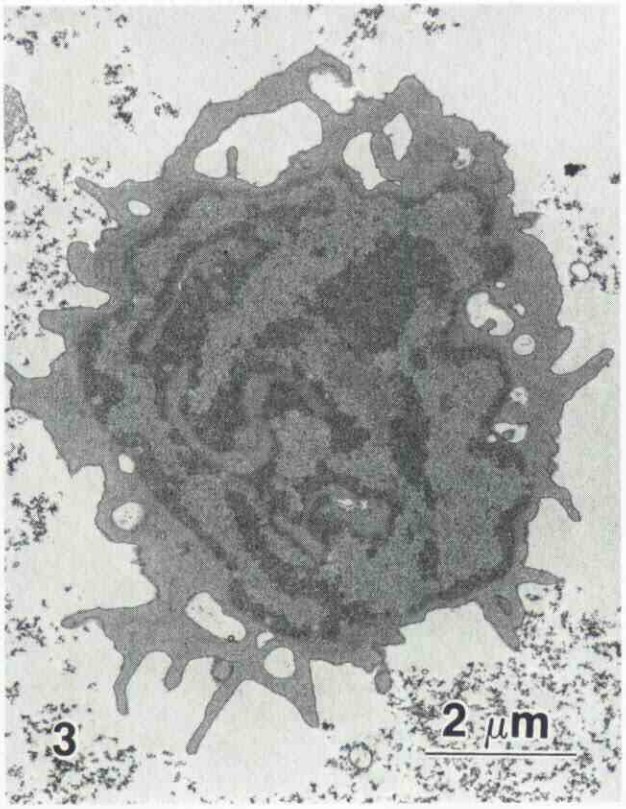


Figure 3. Dark reaction of HUT 102 cell with 8-MOP. Note the extensive cytoplasmic sinuses and the virtual absence of microvilli. Magnification ×9900.

blebs, and multilamellar fungiform membrane evaginations were repeatedly observed in most cells (Fig 4*a,b*). Nonetheless, outside of the damaged membrane segments, the remainder of the plasma membrane remained remarkably intact. Apart from swelling of cristae in some mitochondria, no drastic morphologic effect of 8-MOP on other cell organelles was detected. As was the case in UVA irradiated cells, the cell nuclei exhibited accumulation of heterochromatin along the nuclear envelope but otherwise they remained unremarkable.

A relatively small number of cells (1%) have developed well-formed uropods and have exhibited massive polarization of the cytoplasm. Characteristically the crescent-like appearance of massively polarized cytoplasm was the morphologic hallmark of these cells.

The most diligent search for the analogously transformed cells in control and in UV-A irradiated cultures failed to reveal any.

Effect of UV-A Light and 8-MOP on the Ultrastructure of HUT 102 Lymphoblasts Unlike UV-A irradiated cells and cell cultures after dark interaction with 8-MOP, the combined effect of 8-MOP and UVA was lethal to approximately 50% of the cells as assessed by trypan blue exclusion.

Consequently, PUVA-exposed cell cultures contained numerous dead cells in the various stages of cytolysis. The plasma membrane of putatively viable cells, although remaining essentially intact over most of the cell circumference, invariably displayed fungiform multilamellar evaginations and was also decorated with membrane-associated round extracellular bodies (Fig 5*a,b*). Although the nature of ultrastructural alterations induced by PUVA generally replicated structural alterations brought about independently by UVA and by 8-MOP, in some ways their extent was noticeably less pronounced. Thus, most cell organelles after PUVA appeared to be undamaged, the areas of the extensive cytoplasmic damage were smaller and less abundant than in UVA-irradiated cells, and many cells retained at least some of their microvilli. Frequent aggregations of heterochromatin along the nuclear envelope were detected in a number of cells. The presence of uropods and massive polarization of cytoplasm was also registered in approximately 1% of the examined cells (Fig 6*a,b*).

DISCUSSION

Microscopic analysis of structural plasma membrane changes in cells exposed to UVA irradiation, dark interaction with 8-MOP, and PUVA constituted the focal point of our efforts. As far as possible, we intend to correlate the present results with our previous work on cell-membrane photomodification [4,5,11,12], and with the pertinent findings reported by others [1–3,13–15]. With this in mind, HUT 102 cell cultures, originally established from erythrodermic T-cell leukemia [7,8], served as a target for UVA irradiation and for supraphar macologic 8-MOP concentration of 15 μg/ml. Because it was shown that high 8-MOP levels induce readily detectable alterations in suitable targets [4,5,11–13], whereas therapeutic 8-MOP frequently failed to yield unambiguous results [6], we were of the opinion that the retrograde progression from high to low psoralen levels subsequently will make it easier to identify subtle alterations possibly triggered by therapeutic psoralen concentrations.

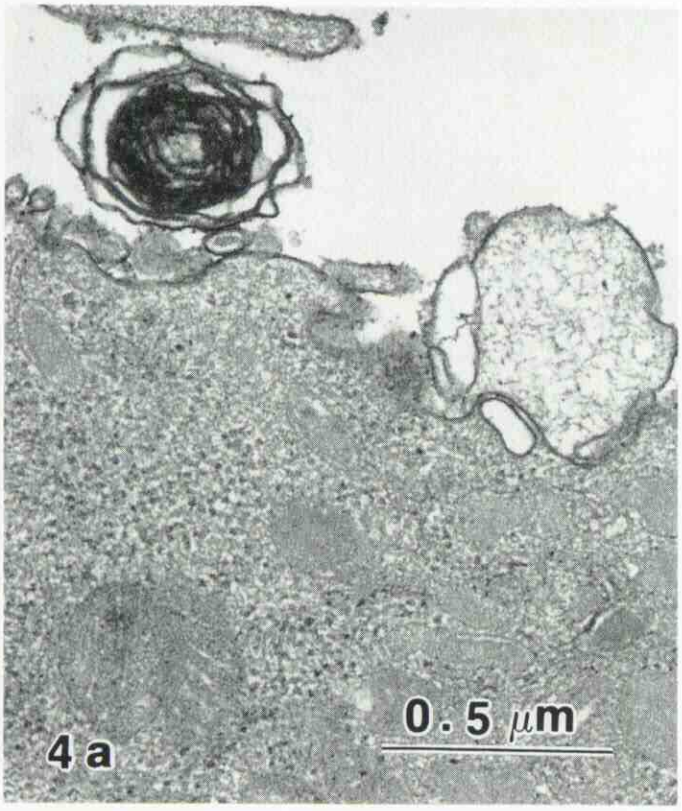
In this study, all morphologic manifestations of cell-membrane damage and alterations induced under stated conditions can be classified as follows: 1) restructuring, retraction, and disappearance of microvilli; 2) the emergence of cell-membrane–associated spherical bodies; 3) formation of the fungiform multilamellar plasma membrane evaginations; 4) formation of uropods and polarization of cytoplasm in some cells. We shall now consider these morphologic categories in the light of pertinent evidence for cell membrane modification by UVA, psoralen, and PUVA.

It seems beyond dispute that sharp numerical reduction, decrease in size, and disappearance of microvilli in response to UVA irradiation, as well as to dark interaction with psoralen, drastically reduces the surface area of target cells. Such radical transformation of plasma membrane topology, almost certainly must result in the rearrangement of plasma membrane receptors, and may well account for the inhibition of EGF [2,3] and lectins [12] binding by PUVA, particularly if the identical results will also be elicited by much lower psoralen levels.

The observed emergence of spheroidal, membrane-associated bodies of unknown function or significance is probably also a membrane-related phenomenon. Because they were identified in all experimental cultures, it may be assumed that their formation was a gradual process, which may have been influenced by our experimental conditions as well as by possibly unique properties of HUT 102 lymphoblasts.

The third category of plasma-membrane damage was expressed morphologically as fungiform multilamellar membrane evaginations and was the direct consequence of independent action on cell membrane by UVA, psoralen, and PUVA. It is noteworthy that these membrane lesions were few per cell cross-section and were occupying spatially restricted membrane segments, thus suggesting the existence of cell membrane loci particularly susceptible to UVA and to psoralen. In view of the evidence that UVA alone also acts on psoralen membrane receptors [2,15], it is tempting to speculate that the fungiform membrane lesions could be the sites of an especially high aggregation of psoralen receptors. Structural degradation of cell-membrane segments adjacent to, and within, the fungiform lesions was sufficiently extensive to allow unimpeded transmembrane trafficking of even large molecules. Indeed, a transient increase of IdUrd incorporation was registered by HUT 102 cells only after the identically implemented dark reaction, but not after UVA irradiation or after PUVA [5].

Similarly, the present study indicates that UVA exerted far greater structural deformation of the cytoplasm than 8-MOP alone or PUVA. By contrast with UVA, extensive lethal photosensitization of HUT 102 cells by PUVA was observed presently and was



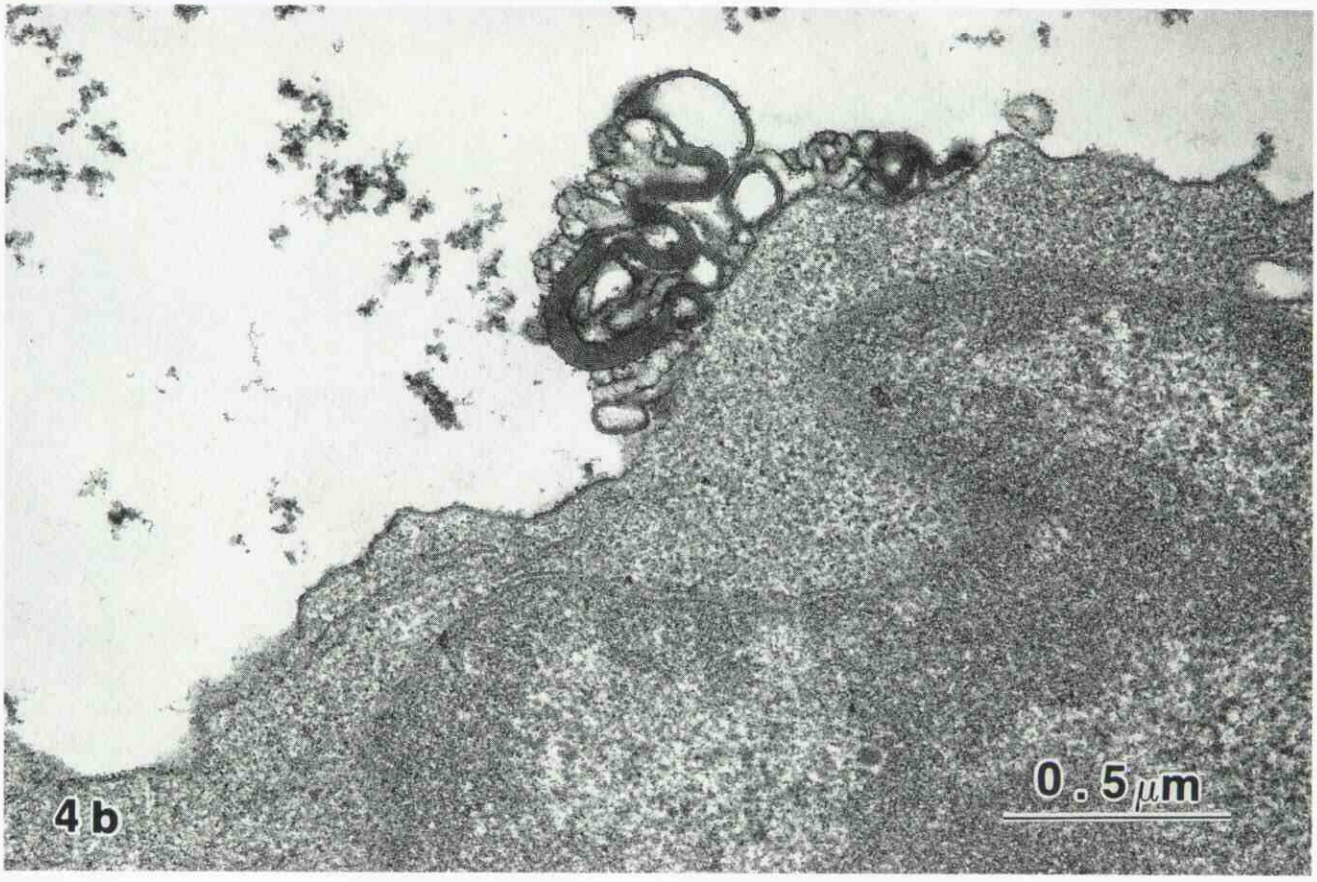


Figure 4. Dark reaction of HUT 102 cell with 8-MOP. *a*: At higher magnification the extracellular body clearly shows its internal lamellar structure. Magnification ×60,000. *b*: An area of an extensive membrane damage reveals sharply defined lamellar periodicity of the fungiform membrane evagination. Magnification ×60,000.

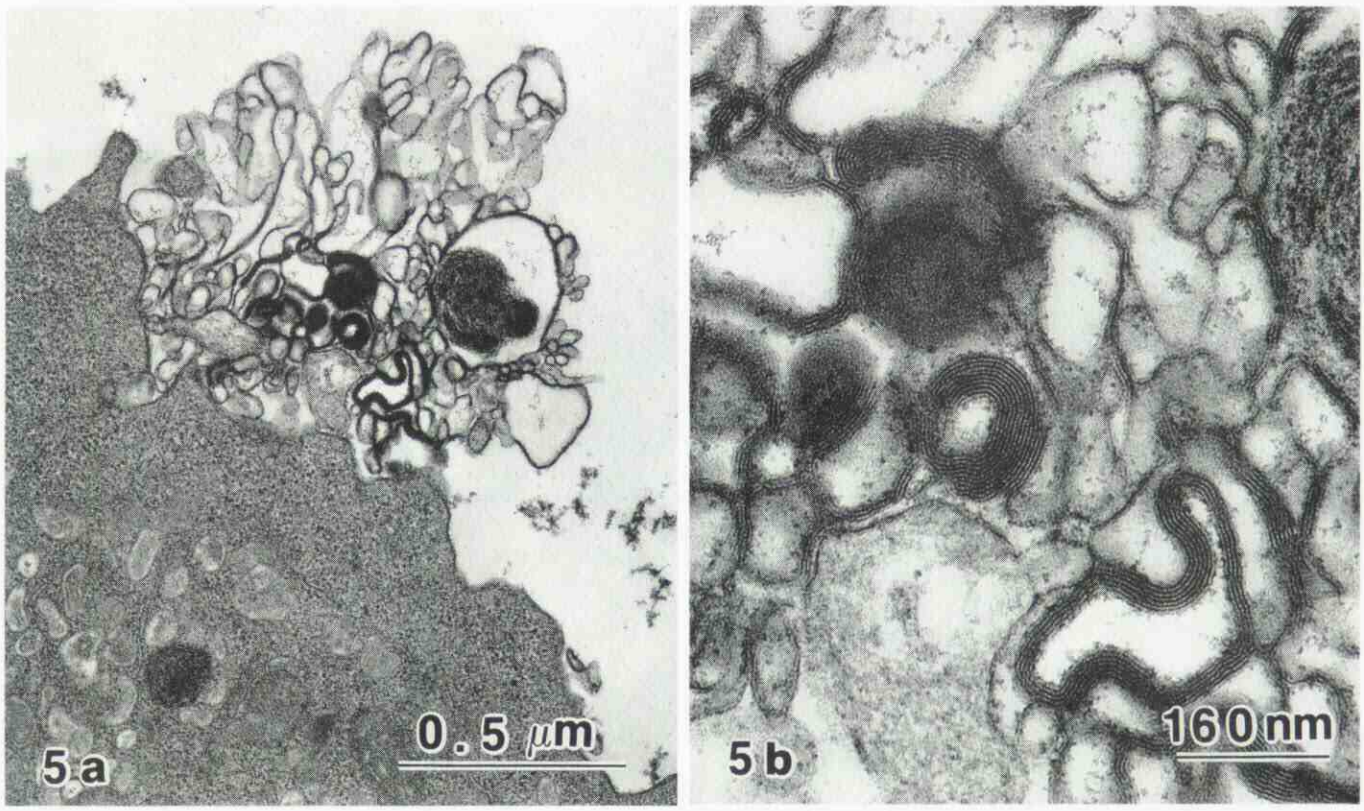


Figure 5. HUT 102 cells after PUVA. *a*: A fungiform multilammellar evagination of an extensively damaged plasma membrane segment. Magnification ×60,000. *b*: The internal structure of fungiform membrane evaginations is clearly evident. Lamellar periodicity is 4.5 nm. Magnification ×126,000.

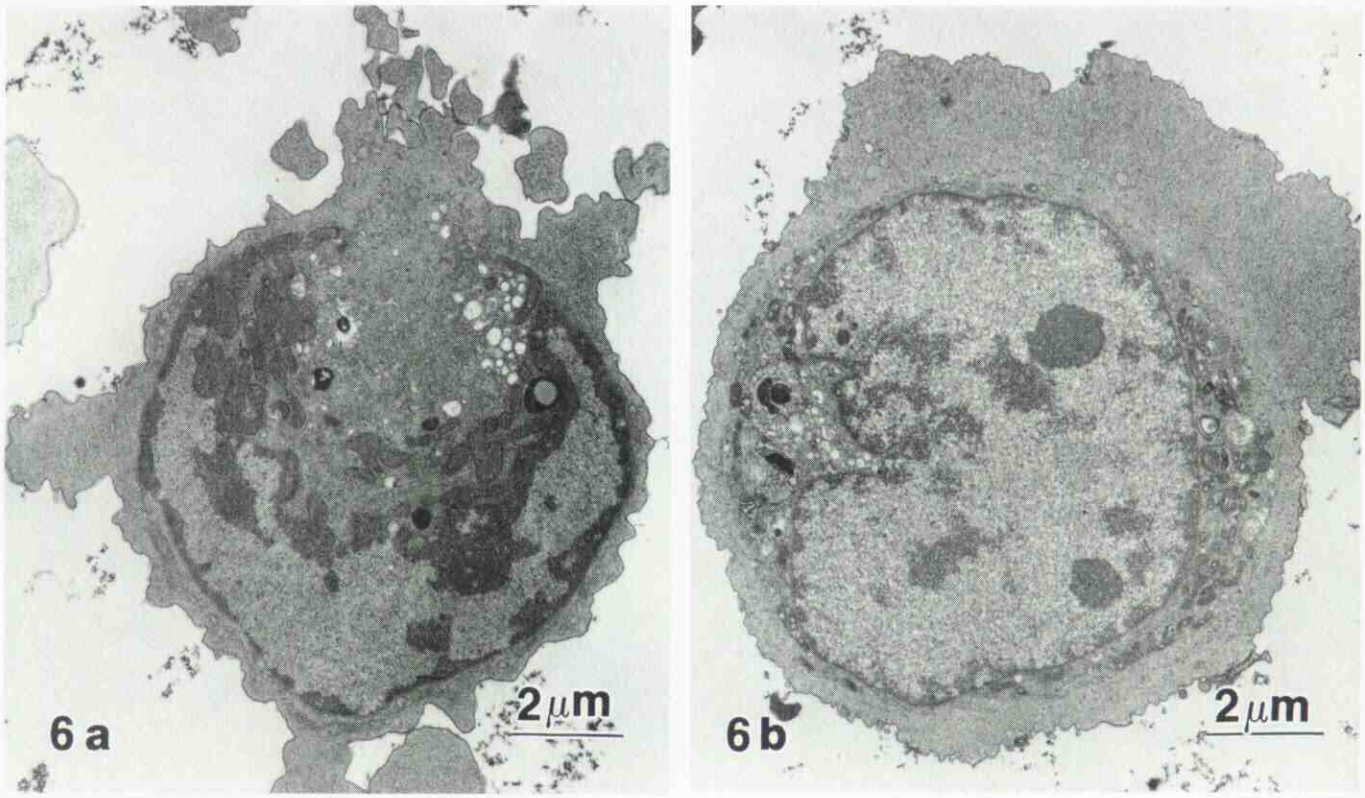


Figure 6. HUT 102 cells after PUVA. *a*: Lymphoblast with tapered uropods and no microvilli. Note the evidently bidirectional polarization of the cytoplasm resulting in nuclear invagination at one pole. Magnification ×7326. *b*: Massive polarization of the cytoplasm in this case resulted in the formation of crescent-shaped cytoplasmic cup. Magnification ×7326.

quantified with respect to the cell cycle in a separate study [5]. We attribute these results to the known capacity of proteins in general [16], and cell membrane proteins especially [11,12], to be cross-linked by UVA and PUVA, thus imparting a degree of structural "rigidity" to cellular components and inhibiting cellular repair mechanisms. Partial retention of microvilli by cells after PUVA may likewise be attributed to the same mechanism. The structural organization of the lamellar membrane lesions was quite similar to the well known myelin figures and therefore it is very likely that beside proteins, membrane lipids are also an integral part of these structures. The well-known lipophilicity of furocoumarins point to membrane lipids as the initial target of 8-MOP. Although the nature of dark psoralen-lipids interactions is unknown, it is the only rational explanation for the consequences of psoralen-induced membrane lesions, during the dark reaction of psoralen with HUT 102 cells and with erythrocyte ghosts [4]. In support of this notion, we note that psoralen-mediated photomodification, of at least some lipid components, was demonstrated [17,18]. Phospholipids, as for example phosphatidylcholine, contain unsaturated and saturated fatty acids, and the former were shown to form photoadducts with psoralen [18]. It seems, therefore, quite possible that lipid-protein cross-linking may be attained under suitable conditions. To the best of our knowledge neither psoralen nor PUVA were ever implicated in cell capping or uropod formation. A few comments pertaining to their possible significance seem, therefore, appropriate.

Uropods were originally defined as cytoplasmic appendages of lymphocytes [19] and were subsequently associated with cell capping [20]. Because cell capping is essentially polarization of cell-surface molecules induced by certain ligands [20], both the cell capping and uropod formation may be viewed morphologically in identical terms. It was well established that spectrin and fodrin, a spectrin-like protein of erythrocyte membrane, are involved in cell capping [21,22] and that spectrin is a major component in electrophoretic band I of human erythrocyte membrane [23,24]. We have also shown that under identical experimental conditions the electrophoretic band I proteins of erythrocyte ghosts are cross-linked during PUVA [11] and that electrophoretic bands I and II of erythrocyte ghosts have selective affinity for ^{3}H-8-MOP [12]. Because the erythrocyte and lymphocyte plasma membrane share a number of proteins [21–24], it stands to reason that they were also involved in psoralen-mediated uropod formation and capping of HUT 102 cells. The same considerations can be applied to the probable role of plasma membrane proteins in the formation of fungiform multilamellar evaginations, and may well explain their structural identicity with the multilamellar erythrocyte ghost lesions [4].

Although plasma-membrane changes and the concomitant cytoplasmic alterations were stressed in this study, the nuclear morphology of HUT 102 cells was also affected by UVA and by PUVA. As was noted previously, the nuclear alterations of HUT 102 cells were qualitatively similar to the analogous morphologic alterations seen in epithelial cell nuclei [25]. The similarity allows us to conclude that response of cell nuclei to PUVA is morphologically more uniform than of cytoplasm and of plasma membrane.

Based on our findings and that of other investigators, we conclude that cell-membrane components are the primary target of 8-MOP and that morphologic changes exerted by PUVA on plasma membranes, to a large extent, replicate alterations induced solely by psoralen.

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