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PSORALEN PHOTOCHEMOTHERAPY OF CUTANEOUS DISORDERS

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HISTORY AND INTRODUCTION

Photochemotherapy is the combined use of a chemical agent with sunlight (or artificial lighting) to induce a medically beneficial result not produced by the chemical or light alone. Psoralens are the most notable of these photosensitizing chemicals. These isomers of furocoumarins (see Figure 1) are found naturally in over two dozen plant sources including *Rutaceae* (e.g. bergamot, lime, cloves), *Umbelliferae* (e.g. celery, parsnip, *Ammi majus*), *Leguminosae* (e.g. *Psoralea corylifolia*), and *Moraceae* (e.g. figs) (1, 2).

Reference to the use of extracts of the *Ammi majus* which grows along the Nile as a weed or seeds of the scurfy pea (*Psoralea corylifolia*) for the treatment of vitiligo can be found in many early medical writings dating back to the *Ebers Papyrus* (ca 1550 BC) (1). It was known that after topical application or ingestion of these concoctions followed by 1–2 hr of strong sunlight, an acute inflammatory reaction with vesiculation would occur. After resolution of this "exaggerated sunburn" response, pigmentation was restored in areas of previous leukoderma (3). Thus, because the sun or plant alone would not produce this effect, photochemotherapy was born.

Pharmacognostic isolation of three crystalline compounds (Figure 1), including 8-methoxypsoralen (8-MOP) in the late 1940s, led to clinical studies in the treatment of vitiligo in the early 1950s (3) and to the development of the synthetic furocoumarin trioxsalen (TMP) in the mid 1960s (2). These agents are now commercially available in the United States for the

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treatment of vitiligo and for inducing photoprotective pigmentation for selected patients.

Success with another photosensitizer in the Goeckerman regimen of topical crude coal tar followed by sunlamp exposure in the treatment of psoriasis (4) along with the vitiligo experience with psoralens led to the

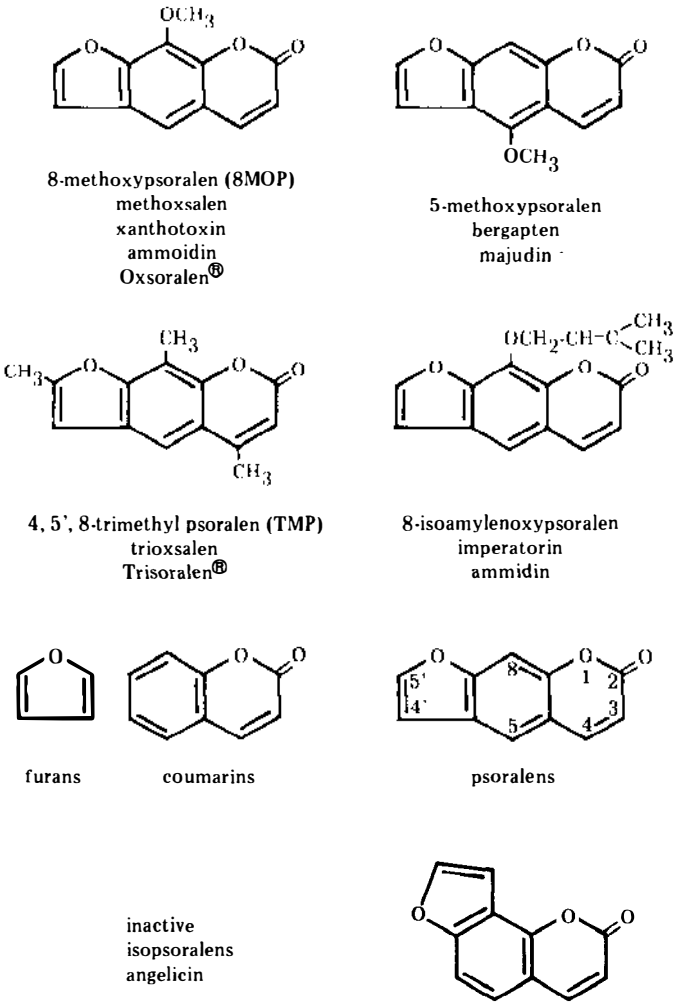

Figure 1 Chemical structure of psoralens.

suggestion as early as 1953 by Lerner et al that a trial psoralen photosensitization for psoriasis should be undertaken (3). This recommendation, along with the 1962 report by Allyn (5) of psoriatic improvement of psoriasis following topical 8-methoxypsoralen and ultraviolet light, was virtually ignored.

Following reports on the photochemistry of psoralens combined with long-wave ultraviolet light (UVA) (Figure 2) (6–8) which resulted in the inhibition of scheduled DNA synthesis in mouse epidermis (9) and in human fibroblasts (10), Walter et al (11) confirmed that psoralen plus ultraviolet light inhibited epidermal DNA synthesis in vitro. Moreover, almost simultaneously Tronnier & Schüle (12) in Europe and Walter et al (11) in the United States illustrated the effectiveness of topical psoralens followed by ultraviolet light in resolving psoriatic plaques in vivo.

In 1974, Parrish et al (13) reported their encouraging results in 21 severe psoriatics treated with *oral* methoxsalen (8-MOP) followed by UVA. They coined the term *photochemotherapy* to emphasize the necessary interaction of light and drug to obtain the therapeutic response, since patients treated with either alone showed no clinical improvement. A shorter name for this photochemotherapy, PUVA, is an acronym for *p*soralen and *u*ltra*v*iolet light type *A*.

There followed multiple reports recounting 80–100% effectiveness of psoralen photochemotherapy treatment of psoriasis with relatively few side

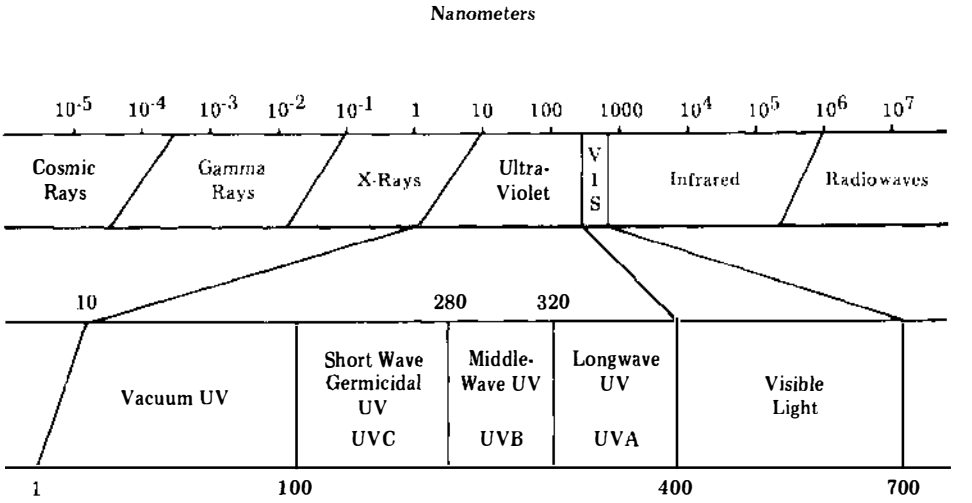

Figure 2 Classification of the ultraviolet portion of the electromagnetic spectrum.

effects utilizing a variety of protocols: oral methoxsalen (8-MOP) plus artificial long-wave ultraviolet light (PUVA) (14–20), oral methoxsalen with sunlight (21, 22), topical methoxsalen with UVA (15, 23), oral troixsalen (TMP) with sunlight (24), and TMP baths with UVA (25, 26). In response to this widespread clinical experimentation, concern has been expressed over the long-term safety of psoralen photochemotherapy (27–31). Although PUVA is unquestionably effective and probably safe for the short term in the treatment of psoriasis, caution must be exercised (limiting the number of patients treated or amount of irradiation delivered) until prospective follow-up can be evaluated.

The initial success of PUVA stimulated much clinical and basic science research. Studies involving photochemotherapy of disorders other than psoriasis; mechanisms of action; adverse reactions and potential risks, comparisons of protocols, techniques, and irradiation equipment; and the use of ultraviolet light alone or with other photosensitizers are being reported in the literature in increasing numbers. Photochemotherapy has come a long way from the ancient Egyptian healers and certainly has a bright future in the expanding field of photobiology.

PSORALEN PHOTOCHEMOTHERAPY RESPONSIVE DISORDERS

Since PUVA was so effective in inhibiting epidermal proliferation in psoriasis, it was postulated that it might suppress proliferating lymphocytes in the cutaneous T cell lymphoma, mycosis fungoides (MF). Results have been promising for the early eczematous patch and plaque stages of the disease with both oral 8-MOP-UVA (32–35) and topical TMP-UVA (36, 37). Disappointing results for the late tumor-nodular stage may be partially explained by histologic examination of PUVA-treated MF lesions. Clearance of the atypical lymphoid infiltrates occurred consistently only above the lower papillary dermis paralleling the depth of ultraviolet light penetration (34, 35). As systemic progression of the lymphoma has been reported despite PUVA (36), photochemotherapy should be considered only as an alternative to the other palliative MF therapies (topical nitrogen mustard, corticosteroids, electron beam therapy).

Other dermatologic disorders responsive to PUVA include such diverse entities as recalcitrant palmoplantar pustulosis (38), atopic eczema (39), lichen planus (40), cutaneous mastocytosis (41), and alopecia areata (42). The induction of ultraviolet light tolerance in polymorphous light eruption (43, 44) and repigmentation in vitiligo (3, 45) continue as the traditional indications for psoralen photochemotherapy.

STUDIES CONCERNING THE MECHANISM OF ACTION OF PSORALEN PHOTOCHEMOTHERAPY

Fundamentals

Many organic compounds absorb radiant energy which then may be utilized in photochemical reactions (46). In biologic systems, chemicals known to participate in these photochemical reactions are photosensitizers. Endogenous photosensitizers known as chromophores include chlorophyll, nucleic acids, aromatic amino acids (e.g. tryptophan), quinones (e.g. melanin), and porphyrins. Exogenous photosensitizers include various tars, certain dyes, tetracyclines, and of course psoralens (47).

The scope of psoralen photosensitized reactions in biologic systems includes erythema and subsequent pigmentation of human and guinea pig skin, death or mutagenesis in bacterial systems, inactivation of DNA viruses, inactivation of enzymes, inhibition of mammalian epidermal cell and fibroblast DNA synthesis, inhibition of the tumor transmitting capacity of various tumor cells, and disorders in the development of sea urchin eggs fertilized by photosensitized sperm (7, 11, 48). The mechanisms involved in all these phenomena are not completely understood, but there has been much work on the effects of psoralens and UVA on DNA, RNA, protein synthesis, epithelial cell membranes, and the immune system.

Effects on DNA

One of the major photochemical reactions possibly initiating the biologic effects of PUVA is the photo-binding of psoralens to DNA. This occurs after irradiation of UVA peaking around 330 nm with a maximum quantum yield at 365 nm (48). These reactions have been characterized as the C_4-cyclo (or cyclobutyl) addition (Figure 3) of psoralen to pyrimidine DNA bases (7). Moreover, two of these reactions lead to interstrand crosslinks formed with native mammalian DNA and photosensitized psoralens (2, 8, 48). It has been suggested that psoralens intercalate between two base pairs of DNA forming a weak association without the presence of ultraviolet light. Then, following UVA irradiation, the 5,6 double bond of pyrimidines can react to either the 3,4 or the 4'5' double bonds of the excited psoralens. Both monofunctional and bifunctional (crosslink) covalent bonds are formed. It is of biologic significance to note that only those psoralen compounds able to crosslink DNA have been shown to have photosensitizing potential. The angular furocoumarins, such as isopsoralen, although capable of intercalating and forming monoadducts, cannot easily crosslink and do not have significant photosensitizing potential (2, 8, 48).

Autoradiographic studies of human fibroblasts exposed to tritium-labeled 8-MOP and UVA indicate a preferential binding of 8-MOP in the nucleus as compared to the cytoplasm (49). Moreover, psoriatics treated with 8-MOP-UVA photochemotherapy showed inhibition of epidermal cell growth using a new "in vivo" H_3-thymidine incorporation autoradiography technique (in which radiolabeled thymidine is injected into the skin prior to biopsy) (50).

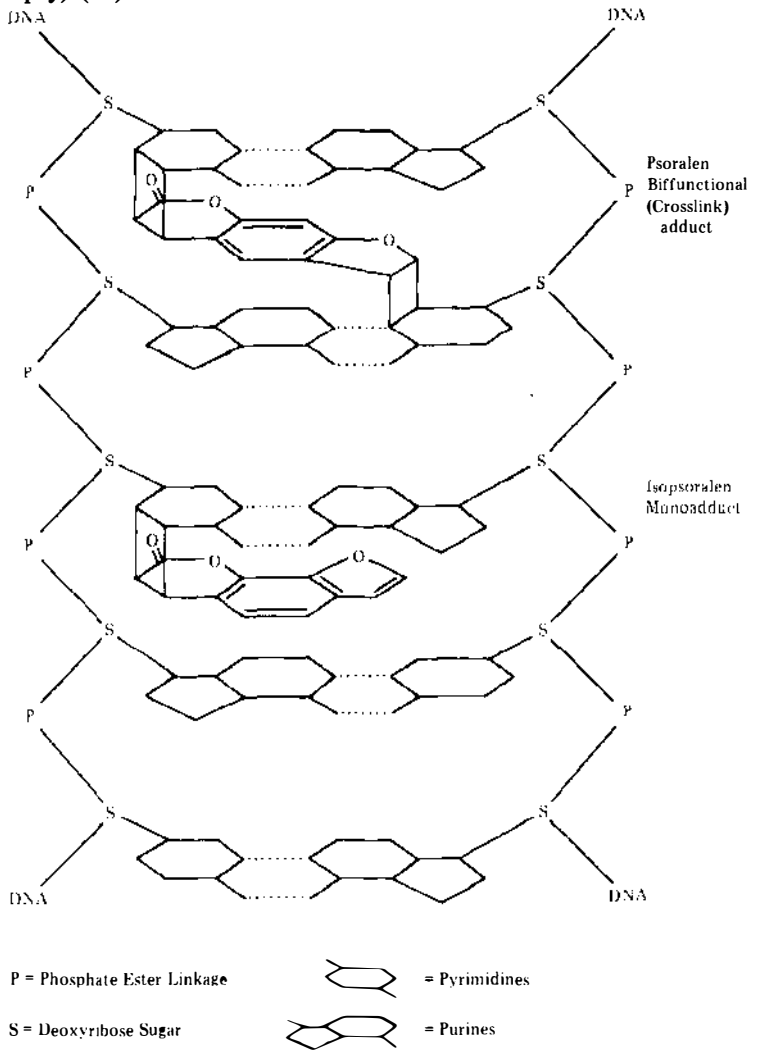

Figure 3 Representation of DNA-psoralen interaction with long wave ultraviolet light.

Psoralen crosslinks have been studied indirectly with ^3H labeling or through their physical properties in vitro (7, 8, 49). Recently direct visualization with electron microscopy of denatured DNA treated in vitro with 8-MOP or TMP and UVA has been reported (51). However, using this technique to examine the skin of psoriatics treated with oral and topical 8-MOP-UVA, no differences in the amount of crosslinking could be found in DNA isolated from PUVA treated dermis, epidermis, or normal human skin removed immediately after irradiation (52). This suggests that while the ability to crosslink may be necessary to be a photosensitizer (2), this action may not be the underlying mechanism in the treatment of psoriasis.

Effects on RNA, Proteins, Cell Membranes, and Organelles

Since the amount of 8-MOP and UVA within the skin can only be estimated, Pohl & Christophers (53) studied dose responses in cultured guinea pig fibroblasts. They were able to produce a fraction of cells incapable of dividing but able to perform other cellular functions such as spreading or attachment. If covalent crosslinking of DNA is considered lethal, then other mechanisms may be playing a role in these photoinactivated cells. Binding and inhibition of RNA has been shown (7, 48, 54), as well as nucleolar changes compatible with DNA-dependent RNA synthesis inhibition and photoalterations in tRNA, with resultant suppression of protein synthesis. Psoralen photobinding to protein without confirmational change has also been shown, but initial damage to mitochondria or lysosomes (implicated in other photosensitivity reactions) has not been observed (54, 55).

Cell membrane damage appears to be the mechanism of porphyrin photosensitization (55). With PUVA, this oxygen dependent "photodynamic type" reaction is kinetically minor compared to DNA cycloadducts (56, 57). However, cultured human glia cells do show cell membrane alteration after PUVA treatment, as revealed by scanning electron microscopy (58). Whether this is primary or secondary to intracellular reactions is unknown. As cell membrane metabolism is very important in the regulation of cellular proliferation (59), it could be speculated that a PUVA-induced alteration of the psoriatic epithelial cell membrane might lead to inhibition of cellular proliferation.

Effects on Cutaneous Erythema

Just as PUVA induces photochemical psoralen adducts to pyrimidines in DNA, it is established that middle wave or "sunburn" UVB (280–320 nm) produces cyclobutane pyrimidine dimers and base alterations in DNA with resultant inhibition of DNA synthesis within 1 hr of exposure (47). Delayed

cutaneous erythema occurs approximately 2–6 hr after UVB irradiation, peaking at 12–24 hr and resolving over 48 hr. PUVA erythema is even further delayed after irradiation with its onset at 24 hr, peaking at 36 to 72 hr, and persisting longer than 7 days (47, 60). Although the link between initial DNA damage and cutaneous erythema may be quite different for UVB and PUVA, knowledge of the mechanisms of UVB erythema may help elucidate the similarities and differences between the two.

Part of the UVB erythema may be due to vasoactive prostaglandin production (PGE_2, PGI_2, etc) (59, 60) within the epidermis or cutaneous blood vessels. Prostaglandins can be shown in sunburned skin by pharmacologic assays, gas liquid chromatography–mass spectrometry, and indirectly by the inhibition of erythema with cyclooxygenase inhibitors such as indomethacin (61). UVB damage to cell membranes may activate phospholipase A_2, releasing arachidonic acid to be metabolized to prostaglandins and other vasoactive oxygenated products (60, 62). As these products may be important in the regulation of cellular proliferation (59), UVB alterations of this system might be part of the mechanism of sunlight or PUVA improvement of psoriasis and other proliferating skin conditions.

It appears that PUVA and UVB erythema differ in more than just time course. While prostaglandin biosynthesis in vitro is affected by 8-MOP alone or in combination with ultraviolet light (63), other studies involving PUVA-treated psoriatic epidermis in vivo have shown no detectable changes in prostaglandin metabolism (64, 65). Moreover, indomethacin does not affect PUVA erythema in contrast to UVB sunburn (60, 66).

Photocutaneous erythema could also be secondary to leucocyte-induced inflammation. Eaglstein et al (67) evaluated cutaneous erythema in cyclophosphamide-treated leukopenic guinea pigs and controls following UVB and PUVA. Only the UVB-treated leukopenic animals showed a significantly altered erythema response. Thus, leukocytes may not be important in PUVA erythema.

Effects on Melanin Pigmentation

Although the mechanisms are unknown, the hyperpigmentation induced by psoralen photochemotherapy involves (*a*) an increase in the number of functional melanocytes (68) within 72 hr after irradiation, and (*b*) an increased tyrosinase activity with concomitant increase in melanin production and formation of melanosomes into proliferating keratinocytes (2, 69). Interestingly, caucasoid skin treated with topical TMP and UVA results in significant alterations in size and distribution of melanosomes toward a Negroid pattern (2). This is apparently *not* the case with systemic PUVA, however (2, 70).

The photoprotective effects of the PUVA tan as compared with control following UVB irradiation (71) have been shown by the inhibition of the erythemic response and by the decreased autoradiographic evidence of unscheduled DNA synthesis (UDS) (reflecting damage and repair). This may well be the mechanism of action in the treatment of polymorphous light eruption (43, 44). The stimulation of melanogenesis by PUVA is also the assumed mechanism in the treatment of vitiligo. However, it is possible that other mechanisms may be playing a role in these two conditions.

Effects on Lymphocytes and the Immune System

It has been suggested that psoriasis and other conditions responsive to PUVA, such as vitiligo, alopecia areata, atopic dermatitis, polymorphous light eruption, and mycosis fungoides, might be pathogenetically based on abnormalities of the immune system (72). Recent studies have shown that both phototherapy with UVB and photochemotherapy may affect this system.

Certainly it is well known that lymphocytes irradiated with UVB in vitro show decreased division or viability, whereas pure UVA alone results in no changes (73). Human lymphoblastoid cell cultures treated with 8-MOP and UVA exhibit DNA crosslinking with exponentially related declining cell survival (74). Significant inhibition of PHA mitogen stimulated proliferation was noted in UVA-irradiated lymphocytes in vitro taken from healthy human donors 2 hr after ingestion of 8-MOP (75). Since it is estimated that 40% of incident UVA penetrates into the dermis (76), it is surprising that the majority (72, 77–80) of in vivo studies have failed to confirm an altered response to mitogens.

Two other studies suggest alternate immunologic grounds for PUVA's mechanism. A leukotactic factor felt to be possibly an immune complex has been extracted from psoriatic scales, and has been proposed for the cause of inflammation and Munro's micro-abscesses seen in active psoriasis (81). PUVA treatments decrease this leukocyte chemotactic activity. On a broader perspective, evidence has been presented to show that mice treated with 8-MOP and UVA are rendered susceptible to UVB-induced tumor formation. These same tumors are readily rejected from normal animals. Since similar observations have been made in mice treated with subcarcinogenic doses of UVB, where immunologic studies have suggested that the tumor-susceptible state is mediated by suppressor T lymphocytes, it is possible that PUVA might induce suppressor T lymphocytes as well. One could then speculate that PUVA might improve proposed autoimmune diseases such as vitiligo, alopecia areata, or polymorphous light eruption through this mechanism (82). Indeed, there is a report of five mycosis

fungoides patients in which PUVA apparently induced a suppression of contact sensitivity to nitrogen mustard (83).

In summary, psoralens intercalate between the nucleotide base pairs of DNA and RNA and interact with long-wave ultraviolet light to form covalent adducts and crosslinks with pyrimidines in vitro. DNA, RNA, and protein synthesis and subsequent cellular proliferation is suppressed both in vitro and in vivo as a result of PUVA. The ultimate psoralen photochemically induced reactions with the cellular metabolism of melanocytes, epidermal cells, lymphocytes, blood vessels, and dermal collagen eventually results in erythema, pigmentation, and possibly alterations in immune function. The relative importance of any of these reactions with respect to the therapeutic and adverse effects of posoralen photochemotherapy is speculative.

REPORTED AND THEORETIC ADVERSE REACTIONS TO PUVA

Carcinogenesis

With the effects of psoralen photochemotherapy on skin and lymphoid DNA and possibly the immune system. questions about the induction or promotion of human carcinogenesis have been raised for the past 20 years (84). This is particularly important when the major indications for therapy are nonmalignant processes such as vitiligo or psoriasis.

The relationship of sunlight to epithelial carcinogenesis has been studied extensively utilizing epidemiologic, animal, and cell culture models (84, 85). Thus far the most attractive mechanism involves DNA damage and/or its repair (85). Indeed, indirect evidence suggests that UV-induced cyclobutane pyrimidine dimers can give rise to tumors per se (86). Also, tumor promotion has been recently correlated with the induction of the enzyme ornithine decarboxylase (ODC) just prior to DNA and cellular proliferation (87). A rise in this enzyme has now been associated with UVB irradiation (88).

If the UVB in sunlight can induce and promote carcinogenesis, it is reasonable that PUVA might parallel this phenomenon. Griffin produced tumors in albino mice treated with dosages of 8-MOP 30–100 times that given to humans followed by daily exposures to severely photosensitizing doses of long-wave ultraviolet light (89, 90). Intraperitoneal doses of psoralens or the addition of UVB irradiation produced a tumor incidence of 50–100%, while oral doses led to an incidence of only 20–35%, as compared to no tumors in the control group treated with light or drug alone (89–92). Lower doses of oral 8-MOP and ultraviolet light failed to produce any tumors in one study, even in albino mice (93). If pigmented mice were

treated in the above models, tumors were produced with a lesser frequency with UVB (92) or not at all with UVA irradiation (90, 94).

In a different experimental model of carcinogenesis, preincubation irradiation with "PUVA lamps" has been shown to promote the transformation of baby hamster kidney cells by polyoma virus (95). Unfortunately, the small amount of UVB "contaminating" irradiation (1 mJ/cm_2) produced by these lamps may be sufficient to induce pyrimidine dimers (85) and could have been the promoter. Moreover, rodent cells (as opposed to human cells) are known to exhibit poor DNA repair of UV-induced damage (96) whereas DNA mono- and bifunctional psoralen adducts have been shown to be easily and quickly repaired (75% in 16 hr) in mammalian skin (2).

After all is said and done with in vitro analysis, clinical studies evaluating the risk of skin cancer in photochemotherapy patients will provide the ultimate answers. Two such prospective studies in the early 1960s of 173 and 92 volunteers treated with 20 mg of 8-MOP or placebo at breakfast followed by ad lib sun exposure showed no statistical difference in tumor incidence between the two groups over a one to two year followup (97, 98). Unfortunately the study was not carried further. However, retrospectively, there have been no reported cases of actinic keratoses or epithelial carcinomas in vitiligo patients treated with psoralens and sunlight continuously and followed for up to ten years (2).

As the doses of drug and light presently used in psoralen photochemotherapy are greater than in this earlier work, and as PUVA appears to act both as an inducer and promoter of carcinogenesis, longer-term prospective studies of patients presently being treated need to be undertaken. One such study of 1373 psoriatics treated with PUVA and followed cooperatively at 16 medical centers, released the preliminary results of the first two years of treatment (99). Thirty patients developed 48 basal and squamous cell carcinomas resulting in an observed overall incidence of 2.63 times that expected for an age, sex and geographically matched population. Those at highest risk were patients with the known risk factors of previous X-ray therapy or cutaneous carcinoma. If these patients were separated out, the rest of the patients had *no* significantly higher risk than the matched population. It is of concern, however, that there was a higher than expected proportion of squamous cell carcinomas overall and an excess of these tumors was found in normally "non-sun-exposed" skin (99). Since many psoriatics treated with agents that might be carcinogenic (UVB, coal tar), or immunosuppressive (methoxtrexate, corticosteroids) have not undergone this close scrutiny, further examination of PUVA patients and other psoriatics not treated with PUVA will yield a better idea of the long-term carcinogenic risks of photochemotherapy.

A greater concern perhaps than the easily treatable epithelial carcinomas now reported is the potential risk of malignant melanoma, considering the link between solar radiation and this tumor (100) along with the melanocytic stimulation of photochemotherapy. Fortunately, thus far, there have been no reported cases of malignant melanoma in any clinical studies or in animal models.

Another area related to DNA damage/repair and carcinogenesis is the potential for chromosomal genetic aberration secondary to photochemotherapy. Reports that in vitro PUVA-treated peripheral lymphocytes exhibited an increased frequency of sister chromatid exchanges (101, 102) prompted studies in vivo. Examination of patients' lymphocytes before PUVA, after initial treatments, and as late as six months into therapy, however, revealed no significant chromatid exchange (102–105).

Granulocytic leukemia has been induced in T cell–deficient nude mice treated with UVB irradiation (106) but has not been reported in nonimmune-deficient animals treated with PUVA (89, 90). There is one report of acute myeloid leukemia developing in a 73 year old psoriatic two years after PUVA treatment was discontinued secondary to the development of two squamous cell carcinomas. However, the relationship to photochemotherapy must remain suspect when one considers the patient's age, previous history of another carcinoma, past treatment with irradiation, and the fact that she received only a small dose of psoralen photochemotherapy (1 month-560 J/cm^2) (107).

Cutaneous Aging

Often associated with cutaneous carcinogenesis is actinically induced senile changes in the skin. It is well accepted that sunlight is a major factor in cutaneous aging (108), producing an altered dermis (consisting of decreased insoluble collagen and increased elastin and ground substance) called solar elastosis. Since PUVA produces over a period of time a dry, thin, wrinkled, "weathered" appearing skin, concern over the possibility of premature aging has been expressed (109).

Although histologic examination has revealed no evidence of increased elastosis per se, an increase in acid mucopolysaccharide ground substance can be seen in some psoritatics after chronic PUVA therapy (109). Multinucleate epidermal cells and fibroblasts (110), colloid-amyloid bodies at the dermal-epidermal junction (111), perivascular amorphous substances (112), and vacuolar epidermal-dermal remodelling termed *photosclerosis* (113) have been demonstrated in some PUVA-treated patients. The relationship of these changes to aging or carcinogenesis is unknown.

Cataractogenesis

In Griffin's (89) study of carcinogenesis of albino mice treated with intraperitoneal 8-MOP followed by UVA, it was noted that almost all of the animals developed corneal opacities. Cloud et al (114) noted extensive damage to the cornea, iris, and lens with 80 mg/kg of oral 8-MOP followed by exposure to UVA for 24 hr in albino guinea pigs and less so in pigmented animals. In another study he induced predominantly anterior cortical cataracts in albino mice who survived 4 mg/kg intraperitoneally followed by 10 min of UVA daily for 10 weeks (115). Freeman & Troll (116) found that if guinea pigs were given only 0.5 mg/kg 8-MOP intraperitoneally daily followed by 10 hours/day of UVA for 13 months (dosages comparable to human photochemotherapy) no ocular injury could be found.

Methoxsalen can be quantitatively detected in the lens of rats measured 2.5 hr (117) but not 24 hr (118) after a single intraperitoneal injection of 4–6 mg/kg of 8-MOP in dimethyl sulfoxide. Although no cataracts in humans due to photochemotherapy have been reported to this date (19) it appears justified to have PUVA patients wear UVA-blocking sunglasses on the day of their therapy.

Reported Adverse Reactions

Coincident with PUVA therapy, one case of discoid lupus erythematosis (119), another with disseminated "actinic" granuloma annulare (120), and three cases of bullous pemphigoid have been reported (121, 122). All of these conditions have been considered possible "autoimmune" disorders exacerbated by sunlight. DNA altered by 8-MOP-UVA has been shown to be antigenic (123), and there have been case reports of psoralen systemic and contact photoallergy (124).

The short-term side effects of PUVA treatments include a 10% incidence of erythema, burns, or blisters, a 3% incidence of nausea, and a 1% incidence of headache or dizziness (19). The greatest complaint, however, is pruritus, with an incidence of 14%. This sensation has been reported by patients as varying from a mild "tingling" to a severe "pins and needles" dysesthesia lasting greater than a week. Two photochemotherapy patients have been reported to demonstrate a loss of the histamine-induced axon-flare response and physical findings consistent with a peripheral neuropathy both of which returned to normal after stopping PUVA (125). Ultrastructural examination of PUVA-treated skin suggests stimulation of cutaneous nerve proliferation and intraepidermal extension of free nerve endings. Many of these nerve endings are associated with melanocytes (126). Thus, it may be speculated that both pruritus and pigmentation may be related to PUVA-induced nerve proliferation.

Although uniform pigmentation or tanning is a normal result of photochemotherapy, a number of patients experienced atypical pigmentation. Profuse freckling (127) and nail pigmentation (128) are two such examples. Phototoxic separation of the nail plate from its bed or photo-onycholysis is another complication (129). Other side effects include a worsening of psoriasis (pustular or erythrodermic flares), induction of herpes zoster, leg edema, or folliculitis (16), as well as, miliaria crystallina, hypertrichosis, skin atrophy, and postinflammatory hypopigmentation.

In summary, most reported adverse reactions to photochemotherapy are mild, reversible (pruritus, burns, or nausea), or of a cosmetic nature (pigmentation disturbances, dry skin). However, considering the effects of PUVA on in vitro and animal models, long-term prospective studies are necessary to assess the risks of cutaneous aging, carcinogenesis, or cataractogenesis.

PHOTOCHEMOTHERAPY PROTOCOL VARIABLES

Patient Selection

With all of the foregoing concerns about long-term safety, guidelines have been formulated concerning patient selection for photochemotherapy (31). Patients should have severe cutaneous disease which may be disabling physically, emotionally, or economically and not easily treatable with conventional therapy of lesser risk. Adult patients are preferred with no history of previous arsenic ingestion, ionizing radiation therapy, concurrent pregnancy, or conditions potentially aggravated by PUVA such as lupus, porphyria, certain bullous diseases, and epithelial or melanocytic malignancy. Caution must be exercised with patients exhibiting preexisting actinic damage, fair complexion, sun sensitivity, cataracts or aphakia, severe cardiovascular disease (because of the heat stress of PUVA), or immunosuppression. Moreover, other than for inducing pigmentation, for which very low dosages of light and drug are necessary, this treatment is considered experimental and requires application to the FDA for an investigational new drug exemption.

Patients vary according to pigmentation and sun sensitivity history (19). Fair skin types I and II (those who never tan or tan poorly) require much less UVA and are at higher risk for the development of skin cancer (19, 99). Skin types III and IV (normal and olive pigmentation) require much more irradiation for photosensitization therapy. Dark pigmentation in Spanish-Americans and Blacks (types V and VI) may actually impair UVA photosensitization enough to lead to treatment failure (14, 19). UVA irradiation dosage should be based on skin type, individual photosensitivity testing, and clinical responses.

Psoralen Clinical Pharmacology

The clinical pharmacology of psoralens is another variable in photochemotherapy. Most orally administered psoralens are absorbed well from the GI tract (reaching a maximum plasma concentration in 1 to 3 hr), metabolized in the liver to hydroxylated products or the glucuronate salt, and excreted in the stool and urine. Over 90% of the total dose is excreted in 12 hr with the rest following over several days (2, 130–132). Inconsistencies in assayed blood levels have been noted among four different pharmaceutical preparations of 8-MOP (130–134). However, photosensitivity, although less related to a specific blood level than to skin type, is maximal with respect to peak plasma concentration in individual patients (130, 131, 134–136).

Although capable of inducing liver mixed function oxidases (137) and cytochrome P450 (138) in animal studies, there appears to be virtually no hepatic or renal toxicity to man (2, 19). Ingestion of psoralens with food may not only relieve nausea (19) but may increase bioavailability (139).

Topical psoralens are effective within 1 hr of application, but may be difficult to use, resulting in inadequate, or conversely, extreme or prolonged photosensitization (11, 26). This may be due to differences in penetration kinetics due to variation in the characteristics of the stratum corneum, underlying draining capillaries, or the vehicle (140).

As the erythemal effects of photosensitization may not manifest for 24 to 72 hr, patients are generally told to apply or ingest the psoralen respectively 1 or 2 hr prior to UVA exposure no more than two to three times weekly (11, 15, 19, 26). As there appears to be no significant advantage of three weekly treatments initially, and since cost and irradiation dose is less, PUVA biweekly may be the schedule choice (19). Oral dosages of 0.1–0.3 mg/kg of 8-MOP and 0.2–0.5 mg/kg of TMP for pigmentation disturbances or photoprotection (141) and 0.5–0.7 mg/kg of 8-MOP for treatment of psoriasis, mycosis fungoides etc (19) have been recommended. Topical concentrations vary among studies from 0.03–1.5 mg/100 ml (11, 15, 26). UVA is increased as needed to maintain a trace erythemal response.

UVA Light Sources and Radiation Measuring Devices

The most difficult variable to quantitate in psoralen photochemotherapy is the light equipment. Numerous sources have been used, including unfiltered sunlight (22, 24), filtered sunlight (21), high output UVA fluorescent bulbs alone (16, 18) or filtered (14, 16, 19), low output "black lights" (11), and other special light sources (25, 142, 143). Radiation measuring devices are not standardized either. This makes comparisons of dosimetry nearly impossible (144, 145). Although irradiation measurement recorded in terms of energy is helpful with comparisons, differences in apparatus design,

radiometry, and age of the light source make significant differences. For example, the energy in J/cm^2 is calculated by multiplying the measured "irradiance" in watts/cm^2 times the exposure time in seconds. The "irradiance," however, can be measured over all the wavelengths emitted by the PUVA lamp, between two wavelengths or corrected for the "action spectrum" desired (144), all giving different results. Moreover, the irradiance decays with the usage of the lamps (145) and may affect different wavelengths nonuniformly. Also, "pure" UVA lamps do emit UVB wavelengths (95) which, although small, may contribute to therapeutic and adverse effects (145).

The wavelength distribution and the irradiation dosage varies between phototherapy units (146) and sunlight (147) and is affected by the patient orientation to the source (148) and by the elements such as cloud cover and wind with outdoor exposure (149). Until all these variables are known or the FDA in concert with the Bureau of Radiation Devices sets rational standards of uniformity, problems in ultraviolet light irradiation dosimetry will be experienced (144).

In summary, the treatment variables in psoralen photochemotherapy include interaction of psoralen pharmacology, ultraviolet radiation equipment, and individual patient responses and risk factors. Photochemotherapy should be administered only by specially trained technicians under the close guidance and supervision of physicians, knowledgeable of these treatment variables.

FUTURE OF PHOTOCHEMOTHERAPY

Obviously, since the long-term effects of photochemotherapy are unknown a general principle of treatment has been to keep the total irradiated dose of UVA as low as possible. To that end it has been suggested that combinations of conventional therapy with PUVA might decrease the amount of UVA needed for clearing or remission maintenance of psoriasis. Topical application of coal tars or anthralin (standard regimens in the treatment of psoriasis) suppress epidermal proliferation (150). While little effect can be shown with a combination of topical tar and PUVA therapy, an enhanced therapeutic effect with topical anthralin and PUVA has been demonstrated (151). Unfortunately, this latter combination therapy is not tolerated by patients because of staining and frequent burns.

New photosensitizers such as anthracene (152) which can also be activated by long-wave ultraviolet light might have potential in the future. However, unless these photosensitizers act by mechanisms other than DNA crosslinking, which may be the case for coal tar or anthracene (153), this approach may not be an improvement. Topical corticosteroids and PUVA

combinations have demonstrated rapid results in the treatment of psoriasis (154–156); however, one study suggested a more frequent recurrence rate with the steroid-treated patients (151).

Systemic adjuncts to PUVA therapy using corticosteroids and methotrexate have been suggested (157). Considering the multiplicity of effects on the photosensitization reaction and the immune system, this approach has been reserved for extraordinary cases. However, a new systemic therapy for psoriasis, retinoids, may have a great future in combination with PUVA (158, 159). These retinoic acid derivatives improve psoriasis via an unknown mechanism (160). They are not photosensitizers (160) and as they inhibit polyamine formation, they may subsequently decrease proliferation and may be *anti*-tumor promoters (87). In combination with PUVA in the treatment of psoriasis, dramatic remissions have been obtained with significantly fewer treatments and less UVA irradiation (158, 159).

Psoralen photochemotherapy has certainly stimulated further research in the areas of carcinogenesis, skin aging, and chromosomal damage, and the PUVA reaction is now being used to probe chromatin substructure (161). Moreover, new photosensitizers are being sought for use in treatment of other conditions, such as photodynamic inactivation of recalcitrant verruca (warts) with photoactive dyes (162), or the treatment of malignant tumors with hematoporphyrin photosensitization (163).

Perhaps synthetic agents will be developed that will be activated only by certain wavelengths of light delivered to specific areas of the body with fiber optics and laser technology. Rooted in ancient Egypt, nurtured by its use in the treatment of vitiligo and now psoriasis, psoralen photochemotherapy remains a fascinating concept of treatment with an exciting future.

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Literature Cited

1. Benedetto, A. V. 1977. The psoralens: An historical perspective. *Cutis* 20: 469–71
2. Pathak, M. A., Krämer, D. M., Fitzpatrick, T. B. 1974. Photobiology and photochemistry of furocoumarins (psoralens). In *Sunlight and Man,* ed. T. B. Fitzpatrick, 22:335–68. Tokyo: Univ. Tokyo Press. 870 pp.
3. Lerner, A. B., Denton, C. R., Fitzpatrick, T. B. 1953. Clinical and experimental studies with 8-methoxypsoralen in vitiligo. *J. Invest. Dermatol.* 20:299–314
4. Goeckermann, W. H. 1925. The treatment of psoriasis. *Northwest Med.* 24:229–36
5. Allyn, B. 1962. *Studies on phototoxicity in man and laboratory animals.* Presented at Am. Acad. Dermatol., 21st, Chicago
6. Musajo, L., Rodighiero, G., Colombo, G., Torlone, V., Dall 'Acqua, F. 1965. Photosensitizing furocoumarins: Interaction with DNA and photoinactivation of DNA containing viruses. *Experientia* 21:22–24

7. Pathak, M. A., Krämer, D. M. 1969. Photosensitization of skin *in vivo* by furocoumarins (psoralens). *Biochim. Biophys. Acta* 195:197–206
8. Cole, R. S. 1970. Light induced cross-linking of DNA in the presence of a furocoumarin (psoralen). *Biochim. Biophys. Acta* 217:30–39
9. Epstein, J. H., Fukuyama, K. 1970. A study of 8-methoxypsoralen (8-MOP) induced phototoxic effects on mammalian epidermal macromolecular synthesis in *in vivo*. *J. Invest. Dermatol.* 54:350–51
10. Baden, H. P., Parrington, J. M., Delhanty, J. D. A., Pathak, M. A. 1972. DNA synthesis in normal and xeroderma pigmentosum fibroblasts following treatment with 8-methoxypsoralen and longwave ultraviolet light. *Biochim. Biophys. Acta* 262:247–55
11. Walter, J. F., Voorhees, J. J., Kelsey, W. H., Duell, E. A. 1973. Psoralen plus black light inhibits epidermal DNA synthesis. *Arch. Dermatol.* 107:861–65
12. Tronnier, H., Schüle, D. 1972. *First results of therapy with long wave UVA after photosensitization of skin.* Presented at Int. Cong. Photobiol., 6th, Bochum, Germany
13. Parrish, J. A., Fitzpatrick, T. B., Tannenbaum, L., Pathak, M. A. 1974. Photochemotherapy of psoriasis with oral methoxsalen and long wave ultraviolet light. *N. Engl. J. Med.* 291:1207–22
14. Wolff, K., Fitzpatrick, T. B., Parrish, J. A., Gschnait, F., Gilchrest, B., Hönigsmann, H., Pathak, M. A., Tannenbaum, L. 1976. Photochemotherapy for psoriasis with orally administered methoxsalen. *Arch. Dermatol.* 112:943–50
15. Lakshmipathi, T., Gould, P. W., Mackenzie, L. A., Johnson, B. E., Frain-Bell, W. 1977. Photochemotherapy in the treatment of psoriasis. *Br. J. Dermatol.* 96:587–94
16. Roenigk, H. H., Martin, J. S. 1977. Photochemotherapy for psoriasis. *Arch. Dermatol.* 113:1667–70
17. Siddiqui, A. H., Cormane, R. H. 1979. Initial photochemotherapy of psoriasis with orally administered 8-methoxypsoralen and longwave ultraviolet light (PUVA). *Br. J. Dermatol.* 100:247–50
18. Nietsche, U. 1978. Photochemotherapy of psoriasis. *Int. J. Dermatol.* 17:149–57
19. Melski, J. W., Tannenbaum, L., Parrish, J. A., Fitzpatrick, T. B., Bleich, H. L. 1977. Oral methoxsalen photochemotherapy for the treatment of psoriasis: A cooperative clinical trial. *J. Invest. Dermatol.* 68:328–35
20. Frenk, E., Perroud, H. 1978. La photochimiothérapie du psoriasis: Expérience lausannoise. *Dermatologica* 157:292–93
21. Bruce, W. W., Wagners, L. T. 1977. PUVA: Using filtered sunlight as an energy source. *Cutis* 20:59–63
22. Parrish, J. A., White, H. A. D., Kingsbury, T., Zahar, M., Fitzpatrick, T. B. 1977. Photochemotherapy of psoriasis using methoxsalen and sunlight. *Arch. Dermatol.* 113:1529–32
23. Weber, G. 1974. Combined 8-methoxypsoralen and black light therapy of psoriasis. *Br. J. Dermatol.* 90:317–23
24. Sehgal, V. N., Rege, V. L., Kharangate, V. N. 1978. Treatment of psoriasis: Trioxalen and sunlight. *Int. J. Dermatol.* 17:243–45
25. Fischer, T., Alsins, J. 1976. Treatment of psoriasis with trioxsalen baths and dysprosium lamps. *Acta Derm. Venereol.* 56:383–90
26. Hannuksela, M., Karvonen, J. 1978. Trioxsalen bath plus UVA effective and safe in the treatment of psoriasis. *Br. J. Dermatol.* 99:703–7
27. Parrish, J. A. 1976. Photochemotherapy of psoriasis. *Arch. Dermatol.* 112:35–36
28. Warin, A. P., Carruthers, F. A. 1976. Photochemotherapy in psoriasis. *Clin. Exp. Dermatol.* 1:181–86
29. PUVA. 1978. *Br. Med. J.* 7:2–3
30. Farber, E. M., Abel, E. A., Schaefer, H. 1978. PUVA appraisal. *Br. J. Dermatol.* 99:715–17
31. Epstein, J. H., Farber, E. M. et al 1979. Current status of oral PUVA therapy for psoriasis. *J. Am. Acad. Dermatol.* 1:106–17
32. Lynch, W. S., Martin, J. S., Roenigk, H. H. 1977. Clinical results of photochemotherapy. *Cutis* 20:477–80
33. Hodge, L., Briffa, D. V., Warin, A. P., Gange, R. W., Bleehen, S. 1977. Photochemotherapy in mycosis fungoides. *Br. Med. J.* 2:1257–59
34. Bleehen, S. S., Briffa, D. V., Warin, A. P. 1978. Photochemotherapy in mycosis fungoides. *Clin. Exp. Dermatol.* 3:377–87
35. Lowe, N. J., Cripps, D. J., Dufton, P. A., Vickers, C. F. H. 1979. Photochemotherapy for mycosis fungoides. *Arch. Dermatol.* 115:50–53
36. Molin, L., Skogh, M., Volden, G. 1978. Successful PUVA-treatment in the tumor stage of mycosis fungoides associated with the appearance of lesions in organs other than the skin. *Acta Derm. Venereol.* 58:189–90

37. Fischer, T., Skogh, M. 1979. Treatment of parapsoriasis en plaques, mycosis fungoides, and Sézary's syndrome with trioxsalen baths followed by ultraviolet light. *Acta Derm. Venereol.* 59:171–73
38. Morison, W. L., Parrish, J. A., Fitzpatrick, T. B. 1978. Oral methoxsalen photochemotherapy of recalcitrant dermatoses of the palms and soles. *Br. J. Dermatol.* 99:297–302
39. Morison, W. L., Parrish, J. A., Fitzpatrick, T. B. 1978. Oral psoralen photochemotherapy of atopic eczema. *Br. J. Dermatol.* 98:25–30
40. Ortonne, J. P., Thivolet, J., Sannwald, C. 1978. Oral photochemotherapy in the treatment of lichen planus (LP). *Br. J. Dermatol.* 99:77–88
41. Christophers, E., Hönigsmann, H., Wolff, K., Langner, A. 1978. PUVA-treatment of urticaria pigmentosa. *Br. J. Dermatol.* 98:701–2
42. Weissmann, I., Hofmann, C., Wagner, G., Plewig, G., Braun-Falco, O. 1978. PUVA-therapy for alopecia areata. *Arch. Dermatol. Res.* 262:333–36
43. Gschnait, F., Hönigsmann, H., Brenner, W., Fritsch, P., Wolff, K. 1978. Induction of UV light tolerance by PUVA in patients with polymorphous light eruption. *Br. J. Dermatol.* 99:293–95
44. Parrish, J. A., le Vine, M. J., Morison, W. L., Gonzalez, E., Fitzpatrick, T. B. 1979. Comparison of PUVA and beta-carotene in the treatment of polymorphous light eruption. *Br. J. Dermatol.* 100:187–91
45. Parrish, J. A., Fitzpatrick, T. B., Shea, C., Pathak, M. A. 1976. Photochemotherapy of vitiligo *Arch. Dermatol.* 112: 1531–35
46. Lamola, A. A. 1974. Fundamental aspects of the spectroscopy in photochemistry of organic compounds. See Ref. 2, 2:17–55
47. Parrish, J. A., White, H. A. D., Pathak, M. A. 1979. Photomedicine. In *Dermatology in General Medicine,* ed. T. B. Fitzpatrick, 101:942–94
48. Musajo, L., Rodighiero, G., Caporale, G., Dall 'Acqua, F., Marcioni, S., Bordiu, F., Baccucheiit, F., Bevilocqua, R. 1974. Photoreactions between skin photosensitizing furocoumarins and nucleic acid. See Ref. 2, 23:369–87
49. Bredberg, A., Lambert, B., Swanbeck, G., Thyresson-Hök, M. 1977. The binding of 8-methoxypsoralen to nuclear DNA of UVA irradiated human fibroblasts *in vitro. Acta Derm. Venereol.* 57:389–91
50. Landthaler, M., Hofmann, C., Plewig, G. 1979. *In vivo* autoradiographic investigations of epidermis in psoriatics under oral 8-MOP-UVA therapy. *Arch. Dermatol Res.* 264:114, (Abstr.)
51. Bohr, V., Lerche, A. 1978. *In vitro* crosslinking of DNA by 8-methoxypsoralen visualized by electron microscopy. *Biochim. Biophys. Acta* 519: 356–64
52. Lerche, A., Søndergaard, J., Wadskov, S., Leick, V., Bohr, V. 1979. DNA interstrand crosslinks visualized by electron microscopy in PUVA-treated psoriasis. *Acta Derm. Venereol.* 59: 15–20
53. Pohl, J., Christophers, E. 1978. Photoinactivation of cultured skin fibroblasts by sublethal doses of 8-methoxypsoralen and long wave ultraviolet light. *J. Invest. Dermatol.* 71:316–19
54. Mizuno, M., Tsuneishi, S., Matasuhashi, S., Kimura, S., Fujimura, T., Ushijima, T. 1974. Some aspects on the action mechanism of 8-methoxypsoralen photosensitization. See Ref. 2, 24:389–444
55. Harber, L. C., Baer, R. L., Bickers, D. R. 1974. Fundamental and cellular aspects of cutaneous photosensitivity reactions (cell membrane changes). See Ref. 2, 21:319–33
56. Poppe, W., Grossweiner, L. I. 1975. Photodynamic sensitization by 8-methoxypsoralen via the singlet oxygen mechanism. *Photochem. Photobiol.* 22: 217–19
57. Dall 'Acqua, F., Magno, S. M., Zambon, F., Rodighiero, G. 1979. Kinetic analysis of the photoreaction (365 nm) between psoralen and DNA. *Photochem. Photobiol.* 29:489–95
58. Wennersten, G. 1979. Membrane damage caused by 8-MOP and UVA-treatment of cultivated cells. *Acta Derm. Venereol.* 59:21–26
59. Voorhees, J. J. 1977. Pathophysiology of psoriasis. *Ann. Rev. Med.* 28:467–73
60. Warin, A. P. 1978. The ultraviolet erythemas in man. *Br. J. Dermatol.* 98:473–77
61. Black, A.K., Greaves, M. W., Hensby, C. N., Plummer, N. A., Warin, A. P. 1978. The effects of indomethacin on arachidonic acid and prostaglandins E_2 and $F_{2\alpha}$ levels in human skin 24 h after UVA and UVC irradiation. *Br. J. Clin. Pharmacol.* 6:261–66
62. Moncada, S., Vane, J. R. 1979. Arachidonic acid metabolites and the interactions between platelets and blood-vessel walls. *N. Engl. J. Med.* 300:1142–47

63. Lord, J. T., Ziboh, V. A., Poitier, J., Legget, G., Penneys, N. S. 1976. The effects of photosensitizers and ultraviolet irradiation on the biosynthesis and metabolism of prostaglandins. *Br. J. Dermatol.* 95:397–406
64. Heiligstädt, H., Kassis, V., Weismann, K., Søndergaard, J. 1978. Prostaglandins in PUVA-treated psoriasis. *Acta Derm. Venereol.* 58:213–16
65. Plummer, N. A., Hensby, C. N., Warin, A. P., Camp, R. D., Greaves, M. W. 1978. Prostaglandins E_2, $F_{2\alpha}$ and arachidonic acid levels in irradiated and unirradiated skin of psoriatic patients receiving PUVA treatment. *Clin. Exp. Dermatol.* 3:367–69
66. Gschnait, F., Pehamberger, H. 1977. Indomethacin does not affect PUVA induced erythema. *Arch. Dermatol. Res. Lett.* 259:109–11
67. Eaglstein, W. H., Sakai, M., Mizuno, N. 1979. Ultraviolet radiation-induced inflammation and leukocytes. *J. Invest. Dermatol.* 72:59–63
68. Rosdahl, I. K., Szabo, G. 1978. Mitotic activity of epidermal melanocytes in UV-irradiated mouse skin. *J. Invest. Dermatol.* 70:143–48
69. Agrup, G., Hansson, C., Rorsman, H., Rosengren, A. M., Rosengren, E., Tegner, E. 1978. 5-S-Cysteinyldopa excretion after treatment with 8-methoxypsoralen and UVA light. *J. Invest. Dermatol.* 70:25–26
70. Zaynoun, S., Konrad, K., Gschnait, F., Wolff, K. 1977. The pigmentary response to photochemotherapy. *Acta Derm. Venereol.* 57:431–40
71. Gschnait, F., Brenner, W., Wolff, K. 1978. Photoprotective effect of a psoralen-UVA-induced tan. *Arch. Dermatol. Res.* 263:181–88
72. Morison, W. L., Parrish, J. A., Epstein, J. H. 1979. Photo-immunology. *Arch. Dermatol.* 115:350–55
73. Cripps, D. J., Horowitz, S., Hong, R. 1978. Spectrum of ultraviolet radiation on human B and T lymphocyte viability. *Clin. Exp. Dermatol.* 3:43–50
74. Cohen, L. F., Kraemer, K. H., Waters, H. L., Kohn, K. W., Glaubiger, D. L. 1979. DNA crosslinking and cell survival in human lymphoblastoid cells treated with 8-methoxypsoralen and long wavelength ultraviolet radiation. *Clin. Res.* 27:240A (Abstr.)
75. Scherer, R., Kern, B., Braun-Falco, O. 1977. UVA-induced inhibition of proliferation of PHA-stimulated lymphocytes from humans treated with 8-methoxypsoralen. *Br. J. Dermatol.* 97:519–28
76. Clayton, R., Valdimarsson, H., Fry, L. 1979. The effect of PUVA treatment on circulating lymphocytes. *Br. J. Dermatol.* 100:225–26
77. Kraemer, K. H., Weinstein, G. D. 1977. Decreased thymidine incorporation in circulating leukocytes after treatment of psoriasis with psoralen and long-wave ultraviolet light. *J. Invest. Dermatol.* 69:211–14
78. Wassilwe, S. W. 1978. Stimulation of lymphocytes in patients with psoriasis under photochemotherapy. *Arch. Dermatol. Res.* 263:127–34
79. Harper, R. A., Tam, D. W., Vonderheid, E. C., Urbach, F. 1979. Normal T lymphocyte function in psoriatic patients undergoing methoxsalen photochemotherapy. *J. Invest. Dermatol.* 72:323–25
80. Fräki, J. E., Eskola, J., Hopsu-Havu, V. K. 1979. Effect of 8-methoxypsoralen plus UVA (PUVA) on lymphocyte transformation and T cells in psoriatic patients. *Br. J. Dermatol.* 100:543–50
81. Mizuno, N., Enami, H., Esaki, K. 1979. Effect of 8-methoxypsoralen plus UVA on psoriasis leukotactic factor. *J. Invest. Dermatol.* 72:64–66
82. Roberts, L. K., Schmitt, M., Daynes, R. A. 1979. Tumor-susceptibility generated in mice treated with subcarcinogenic doses of 8-methoxypsoralen and long wave ultraviolet light. *J. Invest. Dermatol.* 72:306–9
83. Volden, G., Molin, L., Thomsen, K. 1978. PUVA-induced suppression of contact sensitivity to mustine hydrochloride in mycosis fungoides. *Br. Med. J.* 2:865–66
84. Bridges, B. A. 1978. Possible long-term hazards of photochemotherapy with psoralens and near ultraviolet light. *Clin. Exp. Dermatol.* 3:349–53
85. Black, H. S., Chan, J. T. 1977. Experimental ultraviolet light-carcinogenesis. *Photochem, Photobiol.* 26:183–99
86. Hart, R. W., Setlow, R. B., Woodhead, A. D. 1977. Evidence that pyrimidine dimers in DNA can give rise to tumors. *Cell Biol.* 74:5574–78
87. Marx, J. L. 1978. Tumor promoters: Carcinogenesis gets more complicated. *Science* 201:515–18
88. Verma, A. K., Lowe, N. J., Boutwell, R. K. 1979. Induction of mouse epidermal ornithine decarboxylase activity and DNA synthesis by ultraviolet light. *Cancer Res.* 39:1035–40

89. Griffin, A. C. 1959. Methoxsalen in ultraviolet carcinogenesis in the mouse. *J. Invest. Dermatol.* 32:367–71
90. Hakim, R. E., Griffin, A. C., Knox, J. M. 1960. Erythema and tumor formation in methoxsalen-treated mice exposed to fluorescent light. *Arch. Dermatol.* 82:140–45
91. O'Neal, M. A., Griffin, A. C. 1959. The effect of oxypsoralen upon ultraviolet carcinogenesis in albino mice. *Cancer Res.* 19:911–16
92. Pathak, M. A., Daniels, F., Hopkins, C. E., Fitzpatrick, T. B. 1959. Ultraviolet carcinogenesis in albino and pigmented mice receiving furocoumarins: Psoralen and 8-methoxypsoralen. *Nature* 183: 728–30
93. Pathak, M. A., Molica, S. J. 1978. Ultraviolet carcinogenesis in mice and the effect of 8-methoxypsoralen (8-MOP). *Clin. Res.* 26:300A (Abstr.)
94. Langner, A., Wolska, H., Marzulli, F. N., Jablonska, S., Jarzabek-Chorzelska, M., Glinski, W., Pawinska, M. 1977. Dermal toxicity of 8-methoxypsoralen administered (by gavage) to hairless mice irradiated with long-wave ultraviolet light. *J. Invest. Dermatol.* 69: 451–57
95. Morhenn, V. B., Kaye, J. A. 1979. The effect of 8-methoxypsoralen-plus ultraviolet light on cell-virus interaction: The transforming infection; effect of PUVA on the transformation of baby hamster kidney cells by polyoma virus. *J. Invest. Dermatol.* 72:138–42
96. Cleaver, J. E. 1969. Repair replication of mammalian cell DNA: Effects of compounds that inhibit DNA synthesis or dark repair. *Radiat. Res.* 37:334–48
97. Macdonald, E. J., Griffin, A. C., Hopkins, C. E., Smith, L., Garrett, H., Black, G. L. 1963. Psoralen prophylaxis against skin cancer: Report of clinical trial I. *J. Invest. Dermatol.* 41:213–17
98. Hopkins, C. E., Belisario, J. C., Macdonald, E. J., Davis, C. T. 1963. Psoralen prophylaxis against skin cancer: Report of clinical trial II. *J. Invest. Dermatol.* 41:219–23
99. Stern, R. S., Thibodeau, L. A., Kleinerman, R. A., Parrish, J. A., Fitzpatrick, T. B. et al 1979. Risk of cutaneous carcinoma in patients treated with oral methoxsalen photochemotherapy for psoriasis. *N. Engl. J. Med.* 300:809–13
100. Anaise, D., Steinitz, R., Ben Hur, N. 1978. Solar radiation: A possible etiological factor in malignant melanoma in Israel. *Cancer* 42:299–304
101. Wulf, H. C. 1978. Acute effect of 8-methoxypsoralen and ultraviolet light on sister chromatid exchange. *Arch. Dermatol. Res.* 263:37–46
102. Mourelatos, D., Faed, M. J. W., Gould, P. W., Johnson, B. E., Frain-Bell, W. 1977. Sister chromatid exchanges in lymphocytes of psoriatics after treatment with 8-methoxypsoralen and long wave ultraviolet radiation. *Br. J. Dermatol.* 97:649–54
103. Wolff-Schreiner, E. C., Carter, M., Schwarzacher, H. G., Wolff, K. 1977. Sister chromatid exchanges in photochemotherapy. *J. Invest. Dermatol.* 69:387–91
104. Lambert, B., Morad, M., Bredberg, A., Swanbeck, G., Thyresson-Hök, M. 1978. Sister chromatid exchanges in lymphocytes from psoriasis patients treated with 8-methoxypsoralen and longwave ultraviolet light. *Acta Derm. Venereol.* 58:13–16
105. Brøgger, A., Waksvik, H., Thune, P. 1978. Psoralen/UVA treatment and chromosomes. *Arch. Dermatol. Res.* 261:287–94
106. Eaton, G. J., Custer, R. P., Crane, A. R. 1978. Effects of ultraviolet light on nude mice. *Cancer* 41:182–88
107. Hansen, N. E. 1979. Development of acute myeloid leukaemia in a patient with psoriasis treated with oral 8-methoxypsoralen and long-wave ultraviolet light. *Scand. J. Haematol.* 22:57–60
108. Kligman, A. M. 1974. Solar elastosis in relation to pigmentation. See Ref. 2, 12:157–63
109. Bergfeld, W. F. 1977. Histopathologic changes in skin after photochemotherapy. *Cutis* 20:504–7
110. Omar, A., Wiesmann, U. N., Krebs, A. 1977. Induction of multinucleate cells by 8-MOP and UV treatment *in vitro* and *in vivo. Dermatologica* 155:65–79
111. Hashimoto, K., Kumakiri, M. 1979. Colloid-amyloid bodies in PUVA-treated human psoriatic patients. *J. Invest. Dermatol.* 72:70–80
112. Hashimoto, K., Kohda, H., Kumakiri, M., Blender, S. L., Willis, I. 1978. Psoralen-UVA-treated psoriatic lesions. *Arch. Dermatol.* 114:711–22
113. Pierard, G. E., Ackerman, A. B. 1979. Histopathology of remodelling induced by PUVA in the superficial dermis. *Br. J. Dermatol.* 100:251–56
114. Cloud, T. M., Hakim, R., Griffin, A. C. 1960. Photosensitization of the eye with methoxsalen. I. Chronic effects. *Arch. Ophthalmol.* 64:346–51

115. Cloud, T. M., Hakim, R., Griffin, A. C. 1961. Photosensitization of the eye with methoxsalen. II. Chronic effects. *Arch. Ophthalmol.* 66:689–94
116. Freeman, R. G., Troll, D. 1969. Photosensitization of the eye by 8-methoxypsoralen. *J. Invest. Dermatol.* 53:449–53
117. Lerman, S. 1977. A method for detecting 8-methoxypsoralen in the ocular lens. *Science* 197:1287–88
118. Lerman, S., Jocoy, M., Borkman, R. F. 1977. Photosensitization of the lens by 8-methoxypsoralen. *Invest. Ophthalmol. Visual Sci.* 16:1065–68
119. Domke, H. F., Ludwigsen, E., Thormann, J. 1979. Discoid lupus erythematosus possibly due to photochemotherapy. *Arch. Dermatol.* 115:642
120. Dorval, J. C., Leroy, J. P., Massé, R. 1979. Granulomes annulaires disséminés aprés puvathérapie. *Ann. Dermatol. Venerol.* 106:79–80
121. Thomsen, K., Schmidt, H. 1976. PUVA-induced bullous pemphigoid. *Br. J. Dermatol.* 95:568
122. Robinson, J. K., Baughman, R. D., Provost, T. T. 1978. Bullous pemphigoid induced by PUVA therapy. *Br. J. Dermatol.* 99:709–13
123. Zarebska, Z., Jarzabek-Chorzelska, M., Rzesa, G., Chorzelski, T. 1978. Antigenicity of DNA induced by photoaddition of 8-methoxypsoralen. *Photochem. Photobiol.* 27:37–42
124. Plewig, G., Hofmann, C., Braun-Falco, O. 1978. Photoallergic dermatitus from 8-methoxypsoralen. *Arch. Dermatol. Res.* 261:201–11
125. Jordan, W. P. 1979. PUVA, prurtius, and the loss of the axon flare (letter to the editor). *Arch. Dermatol.* 115:636
126. Kumakiri, M., Hashimoto, K. 1978. Cutaneous nerve stimulation by psoralen-ultraviolet A therapy: An ultrastructural study. *J. Invest. Dermatol.* 70:163–72
127. Bleehen, S. S. 1978. Freckles induced by PUVA treatment. *Br. J. Dermatol.* 99:20
128. Naik, R. P. C., Singh, G. 1979. Nail pigmentation due to oral 8-methoxypsoralen. *Br. J. Dermatol.* 100:229–30
129. Mackie, R. M. 1979. Onycholysis occurring during PUVA therapy. *Clin. Exp. Dermatol.* 4:111–13
130. Herfst, M. J., Koot-Gronsveld, E. A. M., de Wolff, R. A. 1978. Serum levels of 8-methoxypsoralen in psoriasis patients using a new fluorodensitometric method. *Arch. Dermatol. Res.* 262:1–6
131. Thune, P. 1978. Plasma levels of 8-methoxypsoralen and phototosicity studies during PUVA treatment of psoriasis with meladinin tablets. *Acta Derm. Venereol.* 58:149–51
132. Gazith, J., Schalla, W., Schaefer, H. 1978. 8-Methoxypsoralen-gas chromatographic determination and serum kinetics. *Arch. Dermatol. Res.* 263: 215–22
133. Hensby, C. N. 1978. The qualitative and quantitative analysis of 8-methoxypsoralen by HPLC-UV and GLC-MS. *Clin. Exp. Dermatol.* 3:355–66
134. Wagner, G., Hofmann, C., Plewig, G. 1979. 8-MOP-plasma levels in PUVA problem patients with psoriasis. *Arch. Dermatol. Res.* 264:116
135. Thune, P., Volden, G. 1977. Photochemotherapy of psoriasis with relevance to 8-methoxypsoralen plasma level and low intensity irradiation. *Acta Derm. Venereol.* 57:351–55
136. Steiner, I., Prey, T., Gschnait, F., Washüttl, J., Greiter, F. 1978. Serum levels of 8-methoxypsoralen 2 hours after oral administration. *Acta Derm. Venereol.* 58:185–86
137. Mandula, B. B., Pathak, M. A., Nakayama, Y., Davidson, S. M. 1978. Induction of mixed-function oxidases in mouse liver by psoralens. *Br. J. Dermatol.* 99:687–92
138. Tsambaos, D., Vizethum, W., Goerz, G. 1978. Effect of oral 8-methoxypsoralen on rat liver microsomal cytochrome P-450. *Arch. Dermatol. Res.* 263:339–42
139. Ehrsson, H., Nilsson, S. O., Ehrnebo, M., Wallin, I., Wennersten, G. 1979. Effect of food on kinetics of 8-methoxsalen. *Clin. Pharmacol. Ther.* 25:167–71
140. Gazith, J., Schalla, W., Bauer, E., Schaefer, H. 1978. 8-Methoxypsoralen (8-MOP) in human skin: penetration kinetics. *J. Invest. Dermatol.* 71:126–30
141. Fitzpatrick, T. B., Parrish, J. A., Pathak, M. A. 1974. Phototherapy of vitiligo. See Ref. 2, 53:783–91
142. Plewig, G., Hofmann, C., Braun-Falco, O., Nath, G., Kreitmair, A. 1978. A new apparatus for the delivery of high intensity UVA and UVA + UVB irradiation, and some dermatological applications. *Br. J. Dermatol.* 98:15–24
143. Petrozzi, J. W., Barton, J. O., Kligman, A., de los Reyes, O. 1979. Topical mechoxsalen administration and sunlamp fluorescent irradiation in psoriasis. *Arch. Dermatol.* 115:436–49
144. Diffey, B. L. 1978. PUVA: A review of ultraviolet dosimetry. *Br. J. Dermatol.* 98:703–6

145. Roenigk, H. H. 1977. Comparison of phototherapy systems for photochemotherapy. *Cutis* 20:485–89
146. Diffey, B. L., Harrington, T. R., Challoner, A. V. J. 1978. A comparison of the anatomical uniformity of irradiation in two different photochemotherapy units. *Br. J. Dermatol.* 99:361–63
147. Diffey, B. L., Kerwin, M., Davis, A. 1977. The anatomical distribution of sunlight. *Br. J. Dermatol.* 97:407–10
148. Burt, J. E., Luther, F. M. 1979. Effect of receiver orientation on erythema dose. *Photochem. Photobiol.* 29:85–91
149. Owens, D. W., Know, J. M., Hudson, H. T., Rudolph, A. H., Troll, D. 1977. Influence of wind on chronic ultraviolet light-induced carcinogenesis. *Br. J. Dermatol.* 97:285–87
150. Walter, J. F., Stoughton, R. B., DeQuoy, P. R. 1978. Suppression of epidermal proliferation by ultraviolet light, coal tar, and anthralin. *Br. J. Dermatol.* 99:89–96
151. Morison, W. L., Parrish, J. A., Fitzpatrick, T. B. 1978. Controlled study of PUVA and adjunctive topical therapy in the management of psoriasis. *Br. J. Dermatol.* 98:125–32
152. Walter, J. F., DeQuoy, P. R. 1978. Anthracene with near ultraviolet light inhibiting epidermal proliferation. *Arch. Dermatol.* 114:1463–65
153. Pathak, M. A., Biswas, R. K. 1977. Skin photosensitization and DNA cross-linking ability of photochemotherapeutic agents. *Clin. Res.* 25:531A, (Abstr.)
154. Hanke, C. W., Steck, W. D., Roenigk, H. H. 1977. Combination therapy for psoriasis with PUVA and a topical steroid. *J. Invest. Dermatol.* 69:426, (Abstr.)
155. Gould, P. W., Wilson, L. 1978. Psoriasis treated with clobetasol propionate and photochemotherapy. *Br. J. Dermatol.* 98:133–36
156. Schmoll, M., Henseler, T., Christophers, E. 1978. Evaluation of PUVA, topical corticosteroids and the combination of both in the treatment of psoriasis. *Br. J. Dermatol.* 99:693–702
157. Vukas, A. 1977. Photochemotherapy in treatment of psoriatic variants. *Dermatologica* 155:355–61
158. Fritsch, P. O., Hönigsmann, H., Jaschke, E., Wolff, K. 1978. Augmentation of oral methoxsalen-photochemotherapy with an oral retinoic acid derivative. *J. Invest. Dermatol.* 70:178–82
159. Michaëlsson, G., Norén, P., Vahlquist, A. 1978. Combined therapy with oral retinoid and PUVA baths in severe psoriasis. *Br. J. Dermatol.* 99:221–22
160. Goerz, G., Orfanos, C. E. 1978. Systemic treatment of psoriasis with a new aromatic retinoid. *Dermatologica* 157: Suppl. 1, pp. 38–44
161. Wieshahn, G. P., Hyde, J. E., Hearst, J. E. 1977. The photoaddition of trimethylpsoralen to Drosophilia melanogaster nuclei: A probe for chromatin substructure. *Biochemistry* 16:925–32
162. Veien, N. K., Genner, J., Brodthagen, H., Wettermark, G. 1977. Photodynamic inactivation of verrucae vulgares II. *Acta Derm. Venereol.* 57:445–47
163. Dougherty, T. J., Kaufman, J. E., Goldfarb, A., Weishaupt, K. R., Boyle, D., Mittleman, A. 1978. Photoradiation therapy of malignant tumors. *Cancer Res.* 38:2628–35