Diseases of Man Associated with Exposure to Light

MEDICAL GRAND ROUNDS

Parkland Memorial Hospital

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CONTENTS

HISTORICAL NOTE

Ancient man recognized the dominant role of the sun within the ecosphere, and many early cultures responded by assigning it divine status. Perhaps the earliest impetus toward what the Greeks termed heliosis, the controlled exposure of the body to sunlight for promotion of health, emerged from these early ritualistic impulses. If so, the tendency for white skinned people to seek out sunbathing and a so-called healthy-looking tan has remained a persistant delusion throughout much of recorded history. It is ironical that the candidate for board-examinations in internal medicine should be aware of more than twenty causes for epidermal hyperpigmentation, of which nearly all are pathological save pregnancy.

Perhaps the earliest record of photobiologic reactions can be found in the Song of Solomon 1:6. "I am black because the sun has shown upon me." But the earliest observers may have misconstrued the nature of this phenomenon since they were unaware of the role of the invisible ultraviolet portion of the spectrum, Hippocrates' remark "The Scythian race are tawney from the cold, not from the intense heat of the sun" betrays confusion of this sort.

Certain well documented therapeutic effects of ultraviolet, such as induction of synthesis of vitamin D_3 and ameliorating skin diseases such as psoriasis and acne, will be noted below. Of historical interest is the work of Niels Finsen, a nobel prize winner in 1903, who pioneered the successful treatment of lupus vulgaris with ultraviolet light. Despite these uses, most observers today view exposure to sunlight, particularly to the ultraviolet part of the spectrum, as detrimental. Study of the pathogenesis of some of these adverse effects currently forms one of the most exciting and rapidly advancing areas in molecular biology, and will be considered in some detail.

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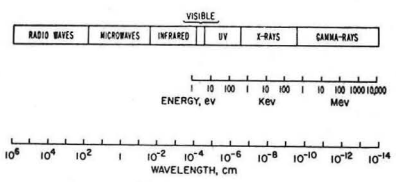
BIOPHYSICAL CONSIDERATIONS

Light and the electromagnetic spectrum: The concept of a continuous electromagnetic spectrum is one of the convenient unities of science. But between high energy radiation (x-ray, gamma, and cosmic rays) and the low energy band (uv, visible and ir) there is a large gap consisting of a stretch of wavelengths not present in nature nor easily reproduced.



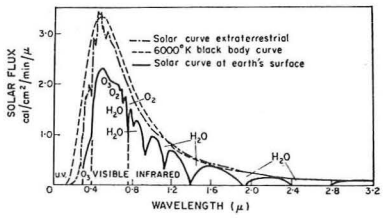
The electromagnetic spectrum.

The first intimation that the solar spectrum extended beyond that range visible to the human eye emerged from the work of William Herschel in 1800. Forming a spectrum with a prism, he passed a thermometer through the various colors and compared each reading with that of a similar, shaded instrument. He was astonished to find that the thermometer showed a higher temperature in the dark space beyond the red than it did in any part of the

visible spectrum. Finding this intense but invisible radiation beyond the red naturally led to a search for a similar phenomenon at the opposite end of the spectrum. Taking advantage of the light-induced blackening of silver chloride, Ritter was able to prove less than a year after Herschel's discovery that the dark portion beyond the violet decomposed silver salts even more rapidly than did visible light.

The first intimation of the existence of radiation beyond the visible violet end of the spectrum was only a few years old in 1814 when Fraunhofer, applying his newly-developed spectroscope to the study of solar radiation, found that the sun's spectrum did not extend beyond a sharp cut-off in the ultraviolet. He detected no bands at wavelengths less than 300 nm. At the turn of the century further observation and theoretical development made it possible to predict the shape of the energy distribution curve outside the atmosphere. By 1921 the absorptive and protective role of atmospheric ozone was understood. Subsequent observations from balloon and rocket vehicles have introduced new refinements in understanding, summarized below.

Solar Energy Distribution: The figure shows two major features of the solar energy distribution. (1) Numerous absorptive bands in the infrared due to water vapor, oxygen, etc. and (2) a major discrepancy due to absorption by ozone in the ultraviolet end. Although available instruments detect fairly strong radiation down to 200 nm in outer space (and at shorter wavelengths when sunspots flare) only miniscule levels of energy below 300 nm penetrate the atmosphere to sea level.



Extra-terrestrial solar flux, the solar flux at the earth's surface, and the flu om a 6 000°K black body as a function of wavelength. The absorption bands resulting fro tmospheric O_2, O_3 and H_2O are shown. (From Gates, 1962.)

Most of this fortunate deficit is due to two overlapping absorptive bands of ozone, in the absence of which the earth could hardly have evolved life as we now know it. The intensly energetic flux of short wave uv light might have made unshaded life impossible. (For purposes of the present discussion, the effects of Rayleigh scattering, aerosol scattering, and smog on the composition of the solar spectrum have been neglected although these effects play an important role in at least one human disease -- the only disorder likely to rest on a deficiency of ultraviolet radiation -- rickets). If one magnifies the important region near the ultraviolet cut-off point, one obtains the following description, emphasizing the effects of the overlying atmospheric mass. This atmospheric factor accounts for more intense ultraviolet flux at high altitudes than at sealevel and for lesser exposures during winter or at higher latitudes when the slanting path followed by the sun's rays duplicates the effect of greater effective air masses.

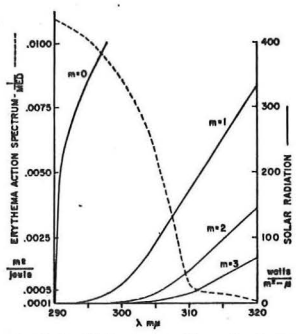
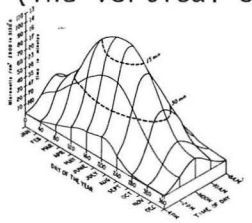
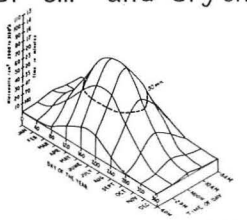


FIG. 3. A rapid decrease in efficiency of erythema production coincides with a rapid increase in intensity of solar energy in the range 290 to 320 mμ (see text for explanation of air mass and units given).

Daniels (5) has reduced a number of complexities to a pair of 3 dimensional figures illustrating the effects of season and hour on minimal erythema time. The figure on the left below, calculated for 30 degrees north latitude, the level of Beaumont, Texas and New Orleans, shows that exposure leads to erythema on white skin in 15 minutes or less between 11 AM and 1 PM in summer, and within 30 minutes during March-October. The right figure illustrates the shorter period of the year and longer time required at 45 degrees, the level of Minneapolis and northern New Hampshire. (The vertical scale is in microwatts per cm^2 and erythema time in minutes.).

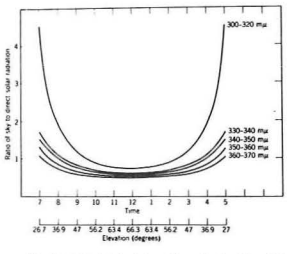


–Minimum erythema time at 30 degrees north latitude; direct sunlight and skylight on a horizontal surface.

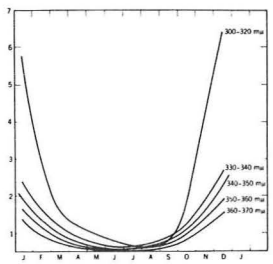


–Minimum erythema time at 45 degrees north latitude; direct sunlight and skylight on a horizontal surface.

Another indication of the complexities introduced by the atmospheric filter emerges if one looks at the ratio of direct radiation to scattered or sky radiation at various times of day and seasons of the year. On a clear day there are many hours during which the amount of ultraviolet from the sky exceeds that following a direct path from the sun, and at all hours it is comparable with that from the sun. These considerations help explain how one can develop a burn even if one is shielded from the direct sun, if one is exposed to a large area of sky.



Diurnal variation in ratio of sky to direct solar ultraviolet radiation. (Calculated from P. Beter, Contract AF 61(052)-618 Technical Note No. 2.)



Annual variation in ratio of sky to direct solar ultraviolet radiation—noon. (Calculated from P. Beter, Contract AF 61(052)-618 Technical Note No. 2.)

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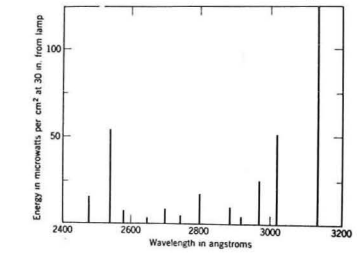
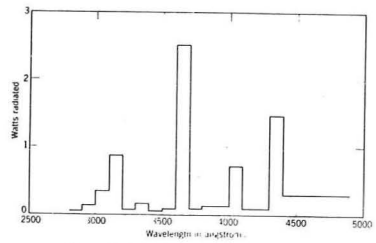
Artificial Sources: One consequence of technological development is that many of us are exposed to sources whose spectral composition differs from that of ordinary sunlight. For example, incandescent lamps radiate almost exclusively in the visible range and can damage tissues only by heat, while daylight fluorescent

tubes produce traces of long-wave ultraviolet, enough to trigger certain photosensitive dermatoses. (7)

Cold Quartz Lamps: Other artificial sources capable of causing photoinjury can be found in industry, laboratory, and in homes. Some of these, such as those containing mercury at low vapor pressure (a few microns) produce radiation characterized by remarkably narrow, discrete lines when analysed by a spectrograph. Such line spectra result from the fundamentally quantized nature of energy; light is ordinarily emitted in packets, or quanta, each with an energy content corresponding to some electronic rearrangement within an excited atom. Only certain transitions of an orbital electron from an excited state to a lower or less energetic state are allowed, or predicted, by the rules of quantum physics. If one restricts the emitting substance to mercury, and further reduces the chances for interfering collisions by keeping vapor pressures within the lamp low, one gets an important fraction of energy emitted as a line at 254 nm, corresponding to the transition of a mercury atom from its lowest excited state to the normal state. Low vapor pressure mercury lamps also operate at low temperatures. They usually are built with quartz envelopes in place of glass in order to allow passage of very short ultraviolet radiation and are referred to as cold quartz lamps. Such devices are used in sterilizing or germicidal applications and, with eye precautions, in certain therapeutic situations in man. Brief exposures to their very short, "unnatural" ultraviolet spectrum produce unusually superficial epidermal injury, often followed by prompt desquamation. The figure shows the energy distribution found in the ideal low pressure quartz mercury arc and emphasizes the importance of the 254 nm line in producing erythema.

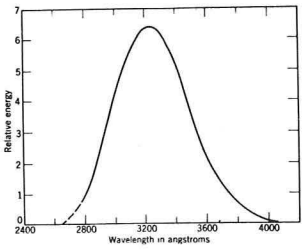




Energy distribution in spectrum of sunlamp 275-RS. (*General Electric Lamp Bulletin* LD-1.)

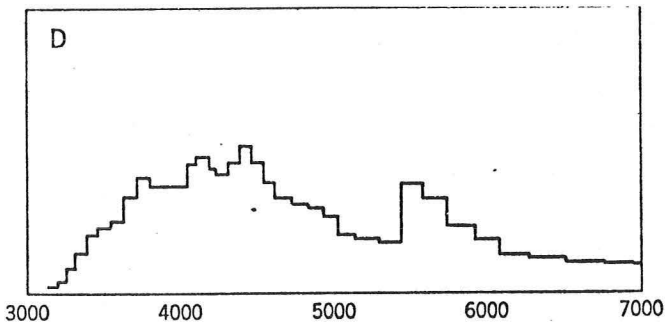
Sun Lamps: Sources of ultraviolet available to the public are known as sunlamps, a term which should be applied only to devices that meet certain criteria established by the council on physical therapy of the AMA. By these standards, the output of such lamps should not differ materially from mid-day sunlight taken in the spectral range 290-313 nm in clear weather, mid-latitude, mid-summer, and at sea level.

The lamp should not emit wavelengths shorter than 280 nm, and at an operating distance of 24 inches it should produce erythema on untanned caucasion skin in a given maximal period. The readily available 275 watt RS sunlamp found in most units intended for the consumer market incorporates an intermediate pressure (2 atm) mercury vapor capillary arc in quartz enclosed within a reflector-type bulb which transmits only radiation above 280 nm. (For technical reasons the glass bulb also contains a tungsten incandescent filament, though its output lies above the ultraviolet range) This lamp radiates sufficient energy between 280 and 320 nm to produce detectable erythema on white skin after approximately 8 minutes exposure at 30 inches distance. As shown in the figure, this lamp also radiates considerable energy at 365 nm and beyond, but these wavelengths lie above the erythema-producing range.



Radiation from E lamps. (H. Froelich, *Trans. Electrochem. Soc.*, **91**, 241 [1947].)

A fluorescent sunlamp is also commercially available. This unit incorporates a phosphor coating on the inner surface of specially treated glass. The glass transmits only wavelengths longer than 280 nm. Within, the lamp contains a low pressure mercury arc as a highly efficient source of 254 nm radiation. The thallum-activated calcium phosphate phosphor absorbs the 254 nm radiation and produces ultraviolet fluorescence over a range of approximately 260 to 400 nm with a peak at 320 nm. The figure illustrates how remarkably the emission of this lamp resembles that of the naturally occurring solar flux below 320 nm. This similarity has led to the use of banks of fluorescent sun lamps as inexpensive sources of solar-quality light for testing and therapy.



Hot Quartz Lamps: Very high partial pressures may be attained in modern mercury arc lamps, pressures at which high temperatures, high current densities, and high operating voltage combine to produce extremely powerful sources of ultraviolet as well as visible light throughout the entire spectrum. At these higher pressures and vapor densities, a mercury arc emits radiation in which narrow line peaks become lower and the continuous bright spectral background increases in importance. Very efficient cooling arrangements are needed for these lamps, some of which reach brightnesses over 200,000 candles per in^2, or 1/5th the brightness of the sun! The largest therapeutic ultraviolet units usually found in physical therapy departments, sometimes used for treatment of total-body psoriasis, contain devices of this type.

Table I. Comparison of Intensity of Ultraviolet

Radiation Delivered by Artificial Sources

	Low Pressure Cold Quartz[1]	Sun Lamp 275 RS	High Pressure Hot Quartz[2]
Lamp watts	30	275	1000
Pressure	few microns	2 atm	110 atm
Watts radiated			
280	8.3	.004	33
280-320		1.4	∿ 56
320-400		3.1	∿ 80
>400		7.5	244

1. Gen. Elec. T_6 germicidal lamp.

2. Gen. Elec. A-H_6 Water-cooled quartz capillary.

Black-Light Lamps: The seemingly paradoxical term black-light refers to radiation of wavelength c. 320-400 nm. This zone of the spectrum possesses several unusual properties; it lies outside the visible range and has trivial or no capacity for evoking erythema. However, light of this region happens to excite fluorescence in a wide variety of naturally-occurring as well as synthetic compounds ranging from psychedelic pigments to those produced by dermatophytes and pseudomonas species.

One may design a black-light lamp in two ways: 1) by enclosing an intermediate pressure mercury arc capillary in a corex glass filter which transmits only in a window in the 330-400 nm region. Essentially the only radiation escaping from such a device is the prominent mercury emission line at 365 nm (refer to figure on page 4). 2) by designing a phosphor coating (similar to that used in the fluorescent sunlamp) capable of radiating in the proper range. Cerium-activated calcium phosphate has the desired characteristics. Just as with the fluorescent sunlamp, a low pressure mercury vapor arc emits large amounts of 254 nm line radiation which the phosphor absorbs and emits as fluorescence with a 360 nm peak.

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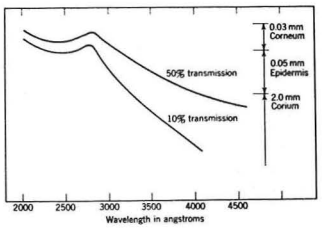
NORMAL RESPONSES OF SKIN TO LIGHT

The complexity of the interactions through which the uv photon passes on its way to the skin surface are small in comparison with the complexity of its optical path once it arrives. Even this path is simple compared with the biologic and biochemical reactions which occur after entry.

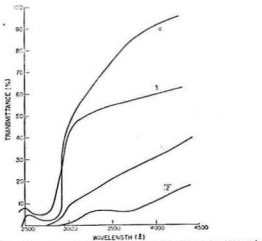
Transfer of Radiation Through Skin: Only radiation of wavelengths less than ~320 nm (particle energy over 4 eV) will produce the erythema of sunburn, while no radiation of wavelength less than 290 nm reaches the earth's surface. Virtually all injurious effects of light in normal persons are thus restricted to the wave band 290-320 nm (exceptions in disease or allergy will be noted below).

The list of variables to be controlled and experimental difficulties to be overcome in measuring light transmittance and reflectance by human skin is dauntingly long. Detailed results may not always be mutually consistent, but a broadly applicable summary of a large body of data follows:

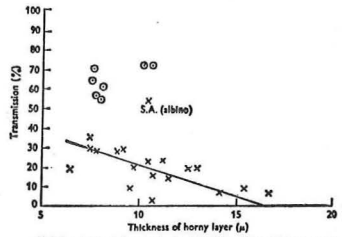
1. Untanned caucasion epidermis transmits most of the 400-1000 nm region but transmission falls rapidly as the wavelength is reduced below 400 nm, and below 300 nm very little is transmitted, although the figures improve if forward scatter is added to that directly transmitted. Much individual variability is encountered, however. One sample tested by Everett et al (13) displayed 7% transmittance at 254 nm, dipped to 5% at 280 nm, and yielded as much as 26% at 297 nm. These results suggest that injurious effects of ultraviolet light on dermal connective tissue may follow direct absorption of damaging wavelengths rather than flowing from release of mediators in the epidermis.



Penetration of human skin by ultraviolet energy. (Adapted from E. Fischer and S. Solomon, *Ultraviolet Radiation*, E. Licht, New Haven, 1959.)



Transmission of erythematous radiation through epidermis. (1) Untanned human epidermis; (2) tanned human epidermis; (3) mouse ear epidermis; (4) human epidermis cleared in acetic acid. (1–3, from Kirby-Smith *et al.*, 1942a; 4, from Lucas, 1930.)



Variation of transmission of solar ultraviolet light (3000 to 4000 angstroms) through the stratum corneum, plotted against thickness of this layer. ○, Europeans; ×, Africans. The numbers in parentheses after initials are percentages for reflectance of blue light on the forearm. (From M. L. Thomson (6))

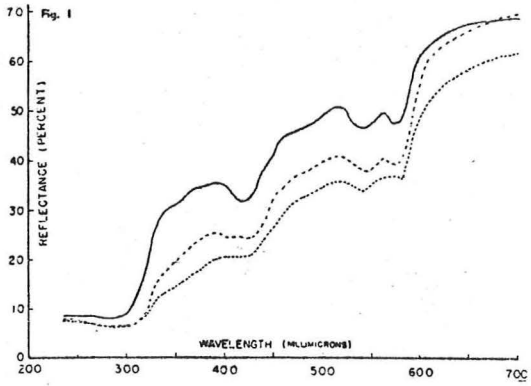
2. Some of the epidermal opacity to uv may be related to scattering in the stratum corneum, for if this layer is cleared with acetic acid or glycerol, ultraviolet transmission rises. This finding suggests that one may be more susceptible to sunburn erythema after prolonged immersion or after use of plastic occlusive dressings (17), or other manipulations which partially clarify the stratum corneum.

3. Besides scattering, some of the sharp cutoff in transmission below 320 nm is due to absorption by aromatic amino acid residues within epidermal protein, a process which is discussed below in greater detail. Lucas (14) showed that both tryptophan and tyrosine solutions absorb maximally at 280 nm mimicing the absorption recorded with cleared stratum corneum. Complete absorption of wavelengths below 250 nm is due to absorption by peptide bonds, while carotene pigments show maximal absorptive effects well outside the erythema-producing range.

4. Another factor accounting for absorption of incident radiation is melanin, capable of absorbing a very broad spectrum. Although little firm data is available, melanin may protect by several mechanisms; it may scatter radiation as well as absorbing it, and may act as a sink for radicals or photoelectrons produced in photochemical reactions in skin. Whatever the mechanism, suntanned caucasian epidermis transmits much less visible radiation, and somewhat less uv radiation, than untanned controls, and heavily pigmented Negro epidermis fillers even more effectively. Thomsom (15) showed that Black epidermis, although no thicker, transmitted less than half as much 300-400 nm radiation as did caucasian.

Table II Absorption Maxima of Some Compounds Present in Skin

Compound	Wavelength	Author
Tyrosine	280	Lucas (14)
Tryptophan		
Carotene	455-485	
Oxy Hemoglobin	350, 417, 548, 578	Edwards (16)
Reduced Hemoglobin	431, 565	
Melanin	300-600 +	



Reflectance of skin

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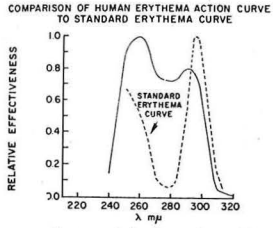
The Erythemogenic Spectrum: Radiation of wavelengths greater than 320 nm can damage skin only through heating; radiation of shorter wavelengths possesses particle quantal energy (>3.9 eV) sufficient to disrupt the molecules individually and is associated with erythema or sunburn. Early attempts to define the spectral curve of erythemal effectiveness were hampered by several factors, including lack of sufficiently intense artificial sources, difficulty in calibrating optical filters, and problems with experimental design. More recently the development of xenon arc devices mated wtih better diffraction gratings have allowed studies with carefully defined wavelengths. The new results do not differ from the older curve in the region accounting for natural sunlight, and still show a slight dip in effectiveness near 280 nm, but clearly document the greater relative effects of more energetic radiation.



Recent quantitatitve data require a modification of human erythema action curve (see text). However, that portion of the curve found in the solar spectrum (290 to 320 mμ) is virtually the same as in the "standard" erythema curve.

Consistent with the reduced epidermal penetration of the shorter wavelengths, erythema produced by the shorter end of the spectrum appears and fades more quickly, and displays a pink rather than red coloration.

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Pathogenesis of Sunburn: The earliest detectable sign of ultraviolet injury to human skin is a reduction in synthesis of protein and RNA, and probably a general decrease in enzyme activity within epidermal cells (21), measurable within minutes of exposure. Indirect evidence suggests that these very early effects first occur in the granular cell and upper prickle cell layer (22) and reach maximal depression at 3 hours, while signs of damage in the basal cell layer remain undetected until 4-12 hours (23).

The details of the pathogenesis of the earliest epidermal damage are still obscure; since the quantal energies available (3.9-4.2 eV) are similar to the energies of C-C and C-N bonds but are less than the ionization potentials for water and peptides, the primary biophysical effect is probably formation of free radicals rather than ions (10). Such radicals may be formed by a single photon mechanism in tyrosine or tryptophan residues within structural or enzymatic proteins. The tyrosine or tryptophan radical may then destroy the integrity of the protein, possibly through disruption of an adjacent cystine-disulfide bond responsible for part of the tertiary structure of the molecule, possibly by peptide bond scission, breaking the continuity of the protein (24). Measurement of electron paramagnetic resonance signals in human skin after ultraviolet irradiation suggests that melanin affects these reactions. Radiation of wavelength less than 320 nm induced a significantly wider free radical signal in white compared with black skin (25).

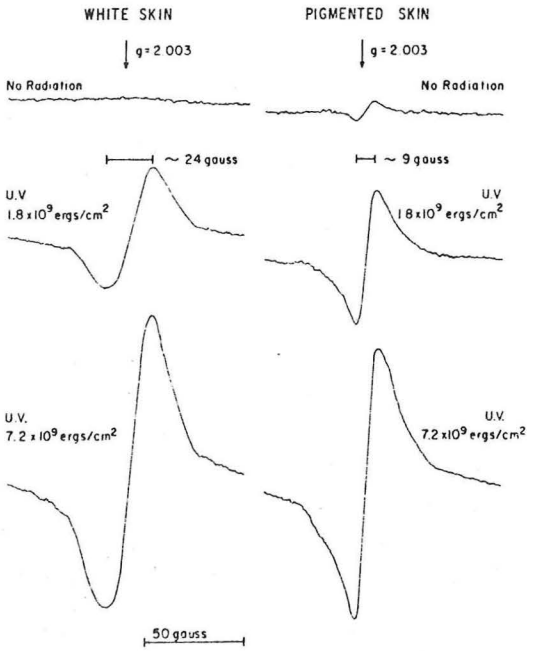

FIG. 2. Electron spin resonance spectra of "white" and pigmented skin at 77°K, before and after ultraviolet irradiation with wavelengths shorter than 320 mμ.

Protein components may not be the sole targets however; an immediate cessation of epidermal mitotic activity seen in irradiated skin of experimental animals suggests that direct damage to nucleic acid may also play a role (26). Potential mechanisms for solar damage to DNA will be discussed below; for the present it will be sufficient to suggest that sunburn may occur through both immediate inactivation of the enzymatic machinery as well as by knocking holes in the template.

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Vasodilatation produces the clinical signpost of erythema, a change often detectable as early as two hours but variably later depending on dose and spectral composition of the inciting radiation. Partington (27) provided the first solid evidence that this long-lasting form of vasodilatation owed little or nothing to histamine release in man, a finding since confirmed in experimental animals (28). More recent studies have suggested several interesting possibilities. A fatty acid-like material with properties resembling prostaglandin was detected in dermal perfusate fluid in 7 of 17 caucasian subjects 24 hours following uv radiation (29) but was not found during the first 8 hours. The finding that aspirin reduces both the discomfort and the redness of sunburn provides further indirect evidence for a contribution to its genesis by prostaglandin (30).

A single report of increased serotonin excretion after uv irradiation in 8 of 10 subjects is difficult to interpret because 5-HIAA excretion did not increase in parallel (31). Using equal exposures (10-12 MED) one group has reported early, transient release of kinin-like substances (32) but other investigators have not detected kinin formation.

In contrast to these uncertainties, the participation of lysosomal hydrolases in the sunburn response seems well established. Not only are lysosomes in mouse skin explants disrupted by uv in vitro, but hydrocortisone substantially prevents uv-induced damage both in a cultured mouse skin system (33) and in human skin in vivo. UV seems to damage the single-layered lysosomal membrane rather selectively, before it affects mitochondrial or plasma membranes (34), but the reasons for this sensitivity are not yet clear. Although these studies were performed with liver lysosomes using 360 nm light, a wavelength which fails to produce cutaneous erythema, there is direct evidence for selective liberation of acid hydrdases at early time points in human epidermis after irradiation with ultraviolet of wavelengths shorter than 320 nm, while no such effects are seen with longer wavelengths (35). Histochemical procedures show particularly dense acid phosphatase activity over pyknotic, condensed cells in the prickle and granular layers, cells that Daniels has chosen to call "sunburn cells", since they appear to be unique to the sunburn reaction.

Lysosomal rupture may also play a role in very early biochemical changes; Halprin's group (36) suggested that lysosomal glucosidase may account for the sharp drop in glycogen content of epidermal cells seen within 3 minutes of uv exposure. It is of interest that this sudden drop sets off a chain of events

leading to massive glycogen accumulation within 12 hours of exposure.

Many phenomena can rather loosely be accounted for if one assumes that lysosomal proteases and hydrolases play a major role in the sunburn reaction. Injected proteases have been shown to cause blistering (37) can split kininogens to pain-causing kinins, and may even remove a blocking peptide to convert tyrosinase from an inactive to an active form (38) leading to enhanced pigmentation. Leukocytic lysosomes appear to cause vasodilatation in other types of inflammatory response; it may be suggested that rupture of epidermal lysosomes may also cause vasodilatation. The fever of severe sunburn may result from systemic absorption of these substances after massive emptying.

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Late Effects of Exposure to Ultraviolet: Epidermal thickening begins within the first 48 hours after exposure, at first due to edema within and between cells,

but due to new cells by the peak in mitotic stimulation at 72 hours (23). When the acute reaction subsides, all layers of the epidermis are thickened and remain so for up to two months after a single exposure. Unlike consequences of the injury produced by heat or other trauma, the response to uv produces a dense, compact stratum corneum which contributes photoprotection in its own right (39). Albinos and patients suffering widespread vitiligo can increase their resistance to ultraviolet to some extent by this means (40). Despite the greater sensitivity of unpigmented skin, the potential of the stratum corneum as a biological filter is illustrated by the practical impossibility of eliciting uv erythema on palms or soles.

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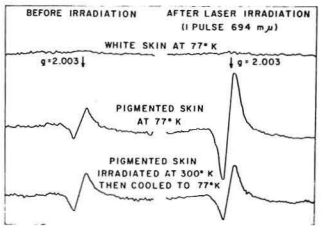
Pigmentation following uv exposure is of two types. First there may be evanescent pigment darkening of melanin granules already within epidermal cells. This reaction can be observed in an already moderately pigmented person or in freckles or moles. It consists of darkening within 5 to 10 minutes of exposure to midday summer sun. The reaction is maximal at an hour and reverses itself in the shade, fading rapidly for the first 10-15 minutes, then more slowly over several hours to days. Only indirect evidence is available on the mechanism of this phenomenon, which may follow exposure to very intense visible light (equivalent to heat) as well as to uv. Based on reversible enhancement of melanin-associated ESR signals, Pathak (41) suggested that increased semiquinone-like free radicals could darken comparatively reduced (brown) melanin polymers to comparatively oxidized (black) ones. The alternate possibility that redistribution of melanin granules within cells can account for this response has been difficult to assess.



Electron spin resonance (E.S.R.) signals from "white" and pigmented skin after laser irradiation.

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The more important delayed type of pigmentation involves production of new pigment within melanocytes, its transfer to epidermal cells, and possible further alteration within such cells on its way to the surface. The process becomes apparent at two days and reaches maximum darkening late in the second week following exposure. Traces of hyperpigmentation may remain for as long as a year, although most of it fades within 3-4 months.

Controversy still surrounds the mechanism of new pigment synthesis due to ultraviolet light (1, 42) although a few certainties have emerged. It is interesting that neither tanning nor racial color differences rest on the presence of greater numbers of melanin-forming cells (43). Instead following uv exposure melanocytes in both blacks and whites produce larger numbers and larger sized melanin granules

are processed in a fashion different from that of smaller granules formed by untanned skin.

These differences in handling of melanin once it reaches the epidermal cell have only recently been recognized. Negro, and caucasoid melanocytes after uv exposure following photosensitization with psoralen (44, 45), produce pigment granules larger than 1 micron in size. Epidermal cells package these large melanosomes individually in pinocytotic vesicles, then in secondary lysosomes, allowing more effective dispersion, and the secondary lysosomes in which they are carried show no tendency to digest their structure. In contrast, unirradiated white skin produces fewer and much smaller granules which are packaged in groups and extensively degraded during their passage through the epidermis (46). Particle size alone apparently determines whether aggregation or dispersion will ensue; epidermal cells engulf experimentally injected latex beads with the same pattern of response (47).

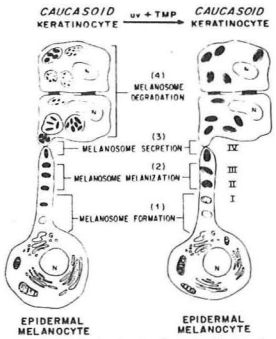

Diagram showing alteration of normal racial pattern in Caucasoid epidermis after TMP application and exposure to longwave ultraviolet irradiation.

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Role of Pigmentation as Protection Against Ultraviolet Radiation: The protective role of epidermal melanin under ordinary circumstances is well established. Early workers showed that the ratio of the thresholds for mild damage in white and negro skin is 1:8; for more severe damage it is much higher 1:120. This greater protection against more severe degrees of damage is also observable in comparing tanned with white, unexposed areas on caucasians. If one considers threshold erythema alone, then increased pigmentation offerslittle protection, while it provides much greater protection from blistering and other important injury (39). In experimental animals skin cancer develops frequently in irradiated albino strains, while their similarly exposed pigmented controls are hardly affected.

The high incidence of cutaneous cancer among albino negroes or those with acquired hypopigmentation has often been noted.

The mechanism of this protective effect is not well understood. As noted above it may protect by absorbing and scattering light and also may form a sink for free radicals or photoactively-produced electrons (1). Seiji (48) has carried out direct measurements of protection afforded subcellular organelles by melanin granules. Using microsomes, mitochondria, and lysosomes isolated from liver he demonstrated that melanosomes confer major protection against direct enzyme inactivation, or in the case of lysosomes, against release of hydrolase, by 254 nm radiation. The precise mechanism by which melanin prevents damage in vivo is as yet unclear, however.

Succinate dehydrogenase activity of mitochondria and of mixture of mitochondria and melanosomes after ultraviolet light irradiation

UV energy 10^5 erg/cm²	Succinate dehydrogenase activity (per cent)	
	Mitochondria	Mitochondria + Melanosomes
0	100	100
1.95	95.9	100
3.9	87.8	94.4
7.8	47.0	90.0
11.7	36.7	73.4

Acid phosphatase activity of lysosomes and of lysosomes + melanosomes after ultraviolet irradiation

Lysosomes non irradiated	Lysosomes irradiated	Lysosomes + Melanosomes (0.5 mg)	Lysosomes + Melanosomes (1 mg)	Lysosomes + Melanosomes (2 mg)
100	177.6	140.6*	130.5*	119*

Lysosomes suspension (4 mg protein/ml) and mixture of lysosomes and melanosomes were irradiated, centrifuged for 20 minutes at 15,000 × g and then the enzyme activity of the supernatant fluid was determined. Enzyme activity is expressed as percentage of the control which is not irradiated.

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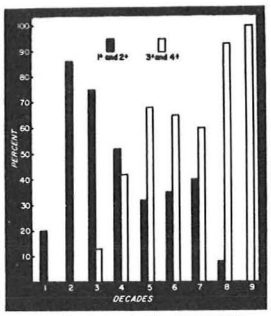
Effects on Connective Tissue: Repeated exposures of caucasoid skin over the years leaves grossly visible changes which include wrinkling, dyspigmentation, telangiectasia, and yellow papules and plaques. The result is a distinctive, furrowed, leathery change often most recognizable on the nuchal area. Specific changes in the dermis include vascular ectasia, accumulation of hyaluronic acid and abnormal appearing fibroblasts, loss of insoluble collagen but an increase in the soluble component, and a marked increase and degeneration in elastin referred to as actinic elastosis.

With the light microscope one can see spectacular changes in affected skin, sometimes in persons in their twenties, with milder but definite damage detectable before age 10 (49). Stains chosen to demonstrate elastic tissue display first an increased number of normal appearing fibers, then fibers that are thickened, curled, and branched, and finally replacement of nearly the full thickness of the dermis by a dense layer of thickened fibers embedded in an amorphous matrix. Essentially no caucasian past age forty has normal dermal elastic tissue in exposed areas, while Blacks maintain a normal or only slightly altered dermis well into the seventh decade. (49) Thus melanin affords substantial, though not quite absolute protection against degenerative changes often mistaken for those of aging, but actually those of actinic exposure. Similar changes have been produced experimentally in animals with exposure to ultraviolet light (51).

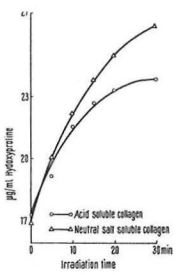


Proportion of persons in each decade showing 1+ to 2+ elastic hyperplasia as compared to 3+ to 4+. In first three decades changes are almost exclusively 1+ to 2+; after fifth decade, 3+ to 4+ changes predominate in each group. By eighth and ninth decade, practically all persons have reached final stage.

The precise origin and composition of the abnormal elastin-like fibers is still debated. Both by light and electron microscopy they resemble elastic fibers, not altered collagen (52). They are refractile, fluoresce appropriately, and resist swelling and dissolution in acids, salts, and bases as true elastin does (53). Elastase digests them but collagenase or purified trypsin does not (54). Their hydroxyproline content is low (53) and their aminoacid content resembles authentic elastin far more closely than it does collagen (55).

Certain results suggest that photochemical alterations in synthesis of new connective tissue proteins by fibroblasts underlies these changes. Recent studies (Magnus and Johnson quoted in (1) and (51) in mice showed that elastic fibers vanished from the skin in the course of repeated exposures but this was followed by the sudden appearance of abnormal elastic tissue after stopping the uv. Large amounts of immature collagen appeared in the dermis simultaneously with the altered elastin. In another study irradiated skin yielded reduced amounts of insoluble collagen with an increase in salt- and acetic-acid soluble collagen (56).

Equally uncertain are the biophysical and biochemical mechanisms that account for these actinically-induced alterations. Besides directly inactivating enzymes and releasing lysosomal hydrolases within epidermis and possibly within dermal fibroblasts, ultraviolet irradiation causes selective depolymerization of hyaluronic acid, without affecting its carboxylic groups, thereby reducing the viscosity of connective tissue ground substance (57, 58). The earliest change may consist of a shift in tertiary structure of polyhyaluronate rather than drop in its molecular weight, since the forces responsible for intramolecular folding are relatively more labile than the covalent bonds between adjacent saccharide units (58). In an intresting but unconfirmed report Raab noted a similar depolymerization-induced fall in viscosity when he irradiated solutions containing soluble collagen. He also detected brisk hydroxylation of bound proline during the radiation experiments, leading him to suggest that uv-induced free e- and hydroxyl radicals can break polypeptide chains as well as hydroxylate susceptible centers (59). Whether sufficient uv intensity reaches the dermis <u>in vivo</u> to produce these very drastic alterations has not been settled. Others have reported unexpected uv-stability of native unpurified collagen (24). Relatively little has been published on changes in other connective tissue components or in their elaboration by fibroblasts following ultraviolet irradiation. Despite this paucity of data the field remains an intriguing and potentially important one.



Increase in hydroxyproline content of acid soluble and neutral salt soluble collagen.

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ABNORMAL RESPONSES OF SKIN TO LIGHT

Neoplastic: Actinic keratoses leading to squamous cell carcinoma and basal cell epithelioma have been etiologically linked at least in part to solar exposure. There are tantalizing suggestions that sunlight may encourage certain forms of melanoma as well, but the evidence is far from conclusive (60). Recent epidemiologic studies (61, 62) have confiemed and extended Dubreuilh's (1907) observations that city workers in Marseilles were less likely to develop skin cancers than were workers in the Bordeaux vineyards (63). Not only exposed occupations but exposed areas are affected first, as would be expected.

Its the absence of exogenous photosensitizers the neoplasia-producing part of the spectrum duplicates the portion of it responsible for inducing ordinary erythema (290-320 nm). But heating effects, humidity, air velocity, and perhaps other factors can increase the harvest of tumors in animal studies, and may alter or enhance human susceptibility as well, possibly as secondary promoters of hyperplasia (64). Planar hydrocarbons of the anthracene class enable the more plentiful longer uv wavelengths to induce tumor formation. There is also evidence that photosensitizing agents such as psoralens taken internally over long periods of time can lower the threshold for long uv-induced tumor formation. The possibility that photocontact dermatitis from topically applied agents, and that a variety of interactions between sunlight and products of our current technology can do the same, should be considered.

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Lack of Protective Pigment: Deficient pigmentation leads to excessive sensitivity to sunburn injury, to grossly visible dermal connective tissue changes at an early age, and to a higher than normal rate of development of cutaneous neoplasms. A classification of genetic forms and clinical features of albinism is presented below (65).

A Tentative Classification of Pigment Mutations in Man

- I. Oculocutaneous Albinism
  - 1. Hair bulb tyrosine test negative
    - a. Tyrosinase negative oculocutaneous albinism
    - b. Albinism-hemorrhagic diathesis (Heřmanský-Pudlak syndrome)
  - 2. Hair bulb tyrosine test positive
    - a. Tyrosinase positive oculocutaneous albinism
    - b. Chediak-Higashi syndrome
    - c. Hypopigmentation-microphthalmia (Cross syndrome)
  - 3. Hair bulb tyrosine test-variable
    - a. Yellow type oculocutaneous albinism
- II. Ocular Albinism
  - 1. X-linked ocular albinism
- III. Cutaneous Albinism
  - 1. Without deafness
    - a. Piebaldism with white forelock
    - b. Questionable isolated white forelock
    - c. Questionable isolated occipital white lock
    - d. Menkes syndrome
    - e. Miscellaneous
  - 2. With deafness
    - a. Waardenburg syndrome
    - b. Cutaneous albinism-hyperpigmentation-deafness Ziprkowski-Margolis syndrome
    - c. Woolf syndrome

Patients with vitiligo, phenylketonuria, or localized loss of pigmentation due to injury or inflammatory skin disease also ran enhanced risks of cutaneous cancer. In the Vogt-Koyanagi-Harda syndrome, a strange, presumably viral disease, the skin and hair around the eyes turns white soon after an episode resembling aseptic meningitis. Occular inflammation leads to photophobia and loss of uv pigment, sometimes accompanied by dysacoria and deafness. Widespread pigment loss reminiscent of vitiligo follows the ocular symptoms. No cause is known, although Fitzpatrick has referred to a cluster of cases in Chihuahua, Mexico suggesting an infectious origin (66).

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Syndromes Combining Telangiectasia with Photosensitivity: Cockaynes Syndrome (67, 68): These children are usually normal until their second year when lagging growth, kyphosis, lipodystrophy of the face, mental retardation, deafness, cere-

bellar ataxia, difficulties with vasomotor tone, retinitis, pigmentosa and light sensitivity develop. The malar regions soon develop mottled pigmentation and atrophic scarring which, along with his loss of facial adipose tissue, give the child a prematurely senile appearance. No studies of the action spectrum have been carried out in this disease. The children usually die in their teens or twenties due to premature atherosclerosis. Limited studies of this rare syndrome have reported a normal karyotype and no apparent susceptibility to chromosome breaks. No studies of DNA repair have been reported.

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Rothmund-Thomson Syndrome (69, 70): Girls outnumber boys 2:1 in this complex developmental syndrome. The essential features of which are (1) onset of photosensitivity in the first or second year with eventual development of mottled pigmentation, atrophy, and telangiectasia resembling radio-dermatitis in exposed areas. (2) sparse hair. (3) high incidence of childhood cataracts (4) small or dwarfed stature, and (5) normal intelligence and life span. No study of the action spectrum has been carried out in these patients, but repair of uv-damaged DNA proceeds normally (71).

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Bloom-German Syndrome (72): Although the separation of this disorder from Cockayne's and the Rothmund-Thompson syndrome has been disputed, their features clearly differ in several important respects. Males predominate 5:1 in Bloom's syndrome, which has been inconclusively assigned as an autosomal recessive. The majority of the patients have been Jewish. Chromosomal abnormalities have been found in essentially all cases. The major features are chronic facial telangietatic erythema (eyelid, malar areas, pinnae) exacerbated by sunlight, moderate, symmetrical retardation of growth, and a relatively high incidence of death from acute leukemia (73). The cutaneous action spectrum appears to fall within the sunburn range (74). These patients appear to have an abnormally high incidence of chromatid breaks in cultured leucocytes, and their susceptibility to both phototoxic skin damage and leukemia has been tentatively linked to this abnormality. However, preliminary studies have exonerated the uv-repair mechanisms for DNA as a feature of the disorder (75).

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A table comparing certain features of several telangiectatic developmental disorders, not all of which exhibit light sensitivity, is added (next page).

HEREDITARY TELANGIECTATIC SYNDROMES
Rothmund-Thomson
Bloom-German
Cockayne's
Ataxia-Telangiectasia
Dyskeratosis Congenita

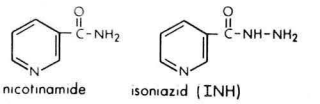
Nutritional Disorders - Pellagra (76): The dermatitis of pellagra may follow mild heat or friction, but often follows exposure to ultraviolet light in the Southwestern United States. The affected areas at first become red and indurated and may later develop bullae and erosions. In the classic adult case the erythema progresses to hyperpigmentation, hyperkeratosis, and a characteristic heavy desquamation, at first revealing normal-appearing pink skin beneath. The face is often affected less severely than the extremities and anterior chest, but the reason for this selectivity is unclear. The borders of the affected area are often prominent on the chest, giving rise to a necklace-like appearance (Casals necklace). The perineal area becomes raw, eroded, and scaly. Stomatitis, gastroenteritis, and neurologic manifestations complete the clinical picture.

Although poverty-stricken over-dependence on a diet of corn or similar sources of protein with low biologic value is less common today than formerly, occasional cases due to straight-forward undernutrition still occur. In parts of the world where kwashiorkor is prevalent it is a common error to attribute to it the cutaneous signs of pellagra. In India one can develop the disease if one must live exclusively on millet (Sorghum vulgare or Jowar), a grain with adequate trytophan but a very high leucine content. One mechanism of the leucine effect may be interference with synthesis of the coenzymeforms of niacin, the di- and triphosphopyridine nucleotides. (77) In developed countries the pellagrin is often a chronic alcoholic, or has a primary illness marked by severe debility or malabsorption, or has a defect in tryptophan utilization or absorption (massive carcinoid tumor) (78), or Hartnup's syndrome. Pellagra-like changes are also occasionally seen following prolonged ingestion of semipurified diets developed for the treatment of inborn errors of metabolism (79), though the tissue changes are probably due to more complex combined deficiencies. Finally a few patients given isoniazid without pyridoxine supplements have developed pellagra (80). Pyridoxine is a required cofactor in synthesis of niacin from tryptophan, but INH may also compete with niacin for incorporation into the active coenzyme diphosphopyridine nucleotide.

HEREDITARY TELANGIECTATIC SYNDROMES

DISORDER	ONSET	SITES	MORPHOLOGY	LIGHT SENSITIVITY	ASSOCIATED FEATURES
Rothmund-Thomson	3 mo - 2 yr	Face, exposed areas (mild changes on unexposed)	erythema telangiectasia mottling atrophy	++	moderate dwarfism sparse hair cataracts
Bloom-German	infancy	face, pinna conjuntivae	erythema telangiectasia	+	moderate dwarfism chromosome breaks leukemia
Cockaynes	2nd yr	face	erythema telangiectasia mottling atrophy	++ bullae	mental and physical retardation retinitis pigmentosa, cataracts
Ataxia-Telangiectasia	3-5 yrs	face, pinna conjunctivae	telangiectasia	--	dwarfism nystagmus IgA deficiency, lymphoma
Dyskeratosis Congenita	5-10 yrs	face	telangiectasia mottling atrophy	--	nail dystrophy oral leukoplakia carcinoma

Modified from Rook, A.S. and Wells, R.S. in Textbook of Dermatology. Davis Philadelphia 1968



nicotinamide isoniazid (INH)

Structural similarities between nicotinamide and isoniazid suggest that INH may inhibit synthesis of DPN by a competitive inhibition mechanism.

Mercaptopurine and presumably azothioprine can also produce pellegrous sunsensitivity, though rarely so (81).

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81. Ludwig, G.D. and D. C. White. Pellagra Induced by 6-Mercaptopurine. Clin. Res. 8:212, 1960.

CONNECTIVE TISSUE DISEASES

LE: The adverse effects of sunlight on both systemic and cutaneous lupus are well established. In large series approximately 1/3 of patients with SLE will have adverse reactions to normally tolerated levels of uv, while twice that fraction of patients with discoid LE will have symptoms (82). Exacerbation of existing skin lesions, fever, and progression of systemic activity may occur after excessive exposure. But sunlight is not an essential part of the pathogenesis of LE. Many SLE patients tan well, occasionally even sustaining accidental sunburn without relapse, though the best medical advice would include instructions for taking reasonable care against overexposure.

Recent use of quantitative phototesting techniques has clarified the pathogenesis of the problem to some extent. These studies indicate that a clinical action spectrum within the sunburn range will produce persistent erythema, indurated plaques, and other LE-like lesions in approximately 1/3 of patients with SLE (83, 84). The older suspicion that such responses represent a nonspecific response to trauma is somewhat difficult to support without restricting the definition of trauma, since very large amounts of long wave uv and visible light (i.e. heat) could be applied to these patients without causing similar lesions (84). Cripps (85) has reported studies that appear to qualify the matter still further. He found that at least one marker for LE-specific inflammatory activity, the band of IgG deposited on the basement membrane between epidermis and dermis, appeared quite late in the course of evolution of a lesion induced by uv light. The ultimate pathophysiologic basis of photosensitivity in LE is as obscure as it was when Casenave first described the disease in 1851, but several possibilities deserve mention.

Tan (86) has used antibody specific for uv-altered DNA to detect DNA photoproducts within the epidermis (and even in the dermis) of irradiated experimental animals. He also showed that uv-irradiated DNA is a potent immunogen, in contrast to the weak antigenicity of native DNA, but that some animals will develop antibodies against native DNA in low titer when appropriately immunized with uv-altered DNA. One group of investigators (87) has found a modest reduction in rate of DNA repair following uv irradiation in LE, and the possibility that delayed repair might predispose these patients to immunization with altered DNA remains to be explored.

The meaning of these results with uv-altered DNA in clinical lupus has yet to be assessed, however. Kunkel's (88) group has documented the close correllation of disease activity with titer of antibodies directed against native DNA both in LE and in the lupus-like disease seen in a high percentage of NZB/W hybrid mice. Interestingly, this group found that 10% of patients with classical active SLE and nephritis did not have detectable anti-DNA antibodies at anytime,hinting that other antigen-antibody systems accounted for their tissue lesions. They were also unable to find antibodies directed against uv-altered DNA in eleven LE patients with documented photosensitivity or in six patients followed serially through episodes of activity. A comprehensive hypothesis is difficult to extract from data as recalcitrant as this. It may be that new approaches will have to be made before a coherent picture of the role of uv in this disorder can be understood.

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REACTIONS DUE TO PHOTOSENSITIZERS

Endogenous: Porphyrins are the only well established photosensitizers synthesized internally. Since disorders marked by excessive synthesis of these compounds, some of which cause severe photosensitivity, have been reviewed at one of these conferences within the past year, they will not be considered in detail. Their adverse effects at the body surface are due to phototoxic enhancement of damage done by light of the long ultraviolet region of the spectrum near 400 nm, well beyond the sunburn zone. This action spectrum correllates closely with the peak of porphyrin absorption at 405 nm (89). The mechanism for this phototoxic effect is likely to involve direct

photoexcitation of the porphyrin molecule to one of its excited states followed by free radical formation and attack on nearby sensitive sites as will be discussed below.

Not commented on at that conference and historically the most interesting of these disorders is the rare recessively-inherited erythropoietic porphyria. Illis (90) has convincingly proposed this disorder as the scientific underpinning for venerable superstitions regarding the existence of werwolves and other mythical fusions of animals and man. These stories were familiar in classical Greece, have been recorded in the literature of five continents, and reached their height during the later middle ages. Illis was able to find several examples of a number of werwolves occurring in the same family. Using medieval and subsequent sources he built up a composite picture of the average werwolf: he was usually an excessively hairy creature with pale greenish or yellowish skin, a red mouth and unsteady eyes. The skin was often described as being covered with sores or excoriations. His behavior was, to put it mildly, disordered. The patient with erythropoietic porphyria fits this description rather well. Being recessive, the disorder was likely to occur in sibs born to consanguinous parents. Sufferers often had red teeth and red urine, suggesting that they feasted on blood. Pallid in hue due to hemolytic anemia, they suffered severe photosensitivity with scarring, hyperpigmentation, mutilation, often followed by hypertrichosis, the whole effect suggesting an animal more than a man. Under the circumstances their tendency to venture forth only at night in a fear-ridden fashion seems understandable, both as a wish to avoid further sundamage and as reluctance to face the suspicions of their neighbors.

TABLE

EFFECTS OF DRUGS IN PORPHYRIA (EALES)

	AIP	VARIEGATE	PCT
Barbiturates	++	++	0
Sulfonamides	++	++	0
Griseofulvin	+	+	+
Estrogens	+	+	+
Iron	0	0	+
Chloroquine	?	?	++
Alcohol	+	+	+

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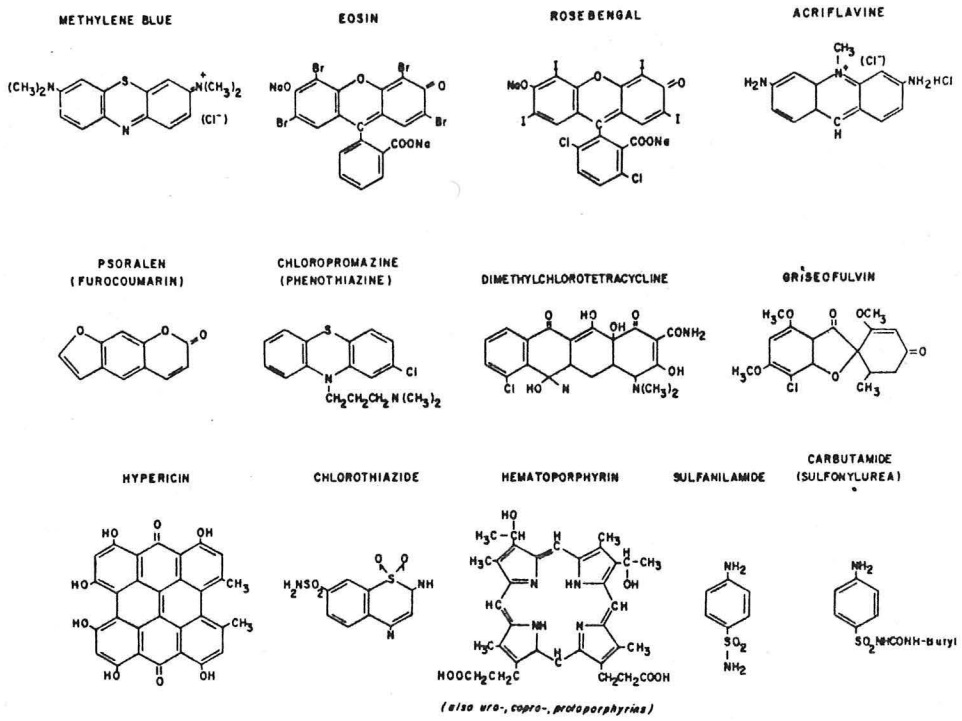
EXOGENOUS

Phototoxic Drug Reactions: The pioneer in this field was Raab whose studies on paramecia in 1900 opened the door to photobiologic principles of critical importance. He noted that acridine orange could be added to the organism's bath in the absence of light without harming them. Exposing them to uv without the dye was also innocuous. However, the dye plus light application caused a lethal reaction for the organism, a reaction which did not occur if the dye was irradiated before adding it to the bath. Oxygen was shortly found essential for the reaction, implying that peroxide formation was involved in the mechanism of damage. The term phototoxic was applied to human medicine in the late 1930's when Epstein observed reactions analogous to those of paramecia after irradiating the skin of patients given intradermal injections of sulfanilamide.

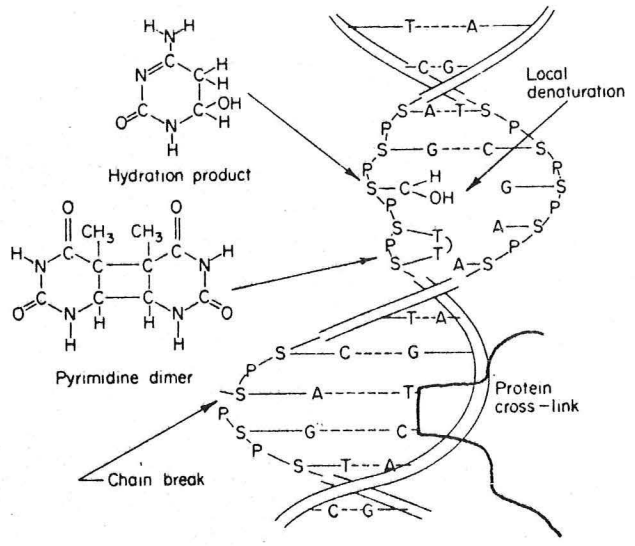
The table presents the major classes of drugs with phototoxic properties and the figure shows that many of these are resonating compounds, most of which are capable of fluorescence.

Major groups of phototoxic agents in man

Sulfonamides	Coal tar
Sulfonylureas	Anthracene
Chlorothiazides	Pyridine
Phenothiazines	Acridine
Tetracyclines	Phenanthrene
Psoralens	



Phototoxic Reactions With Nucleic Acid: The mechanisms by which light and drug interact in a manner harmful to the host have interested several investigators. The possible modes of damage are noted in the figure



Schematic illustration of the various alterations found in DNA extracted from cells that have been irradiated with ultraviolet light. [Adapted from R. A. Deering, *Sci. Am.* 207, 135 (1962).]

Particularly elegant studies have been published which examined interactions of plant-derived psoralens with nucleic acid Unlike other phototoxic drugs, psoralens do not depend upon oxygen for their damaging effects. Musajo (91) showed that a non-damaging loosely associated complex formed when psoralen was added to a solution of DNA in the dark, but when the solution was irradiated with the active longer uv wave lengths, the psoralen reacted covalently to form a 4-carbon, cyclic addition product with pyrimidines, but not purines of DNA. Certain members of the psoralen class also interact with RNA-bound pyrimidines, but the importance of this effect is unclear.

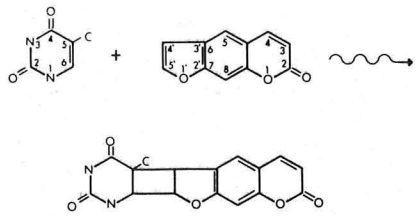


FIG. 3. General type of psoralen photoreaction wi pyrimidine base of DNA. A cycloaddition product

Cole (92) has recently reported that these monoadducts react rapidly and specifically to form diadducts crosslinking the 2 strands of DNA. This second reaction appears to proceed at least as fast as the first, and there is circumstantial evidence to suggest that rates of inactivation in sensitive bacterial and phage systems correlate more closely with rates of formation of these interstrand crosslinks than with the formation of the monoadduct.

REACTION SEQUENCE

Psoralen	+ DNA ⇌	intercalated complex
Complex	+ light (350-400 Nm) ⇌	monoadduct
Monoadduct	+ light ⇌	bifunctional adduct

Trosko (93) has recently shown that the long-wave uv-light-induced psoralen photoproduct severely inhibits the incorporation of ^{3}H-thymidine into DNA of human cells, but does not inhibit, and may stimulate, the repair mechanism concerned with the excision of pyrimidien dimers. Whether or how the psoralen diadduct is repaired is at present unclear.

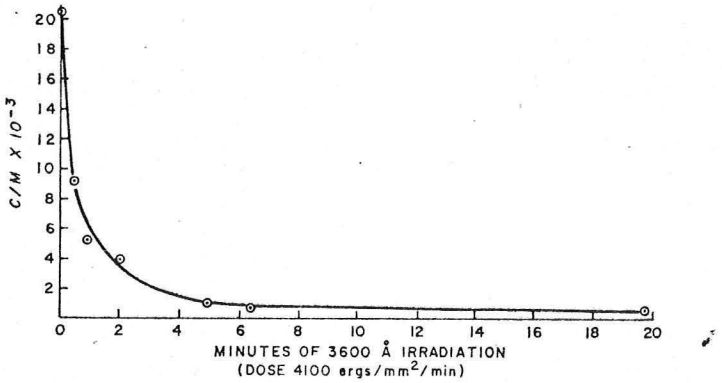


Figure 3. The effect of various doses of 3600 Å radiation on the incorporation of ^{3}H-thymidine into DNA of trisoralen-treated human cells after 4 hours incubation.

It is known that psoralen photo products do not permanently block DNA synthesis. However, DNA synthesized on a template altered by psoralen is of low molecular weight, the length of the newly synthesized pieces corresponding to the average distance between crosslinks (94). Acridine orange and perhaps other phototoxic compounds also have an affinity for the cell nucleus.

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Cytoplasmic Phototoxic Damage: Evidence for interaction of certain photosensitizing compounds with lysosomes has been presented (95) and would correllate with information on the relationship of sunburn erythema to lysosomal rupture reviewed above.

REFERENCE

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Plasma Membrane Phototoxic Damage: Harber and his collaborators (96) have studied the mechanism of photo injury to red cells sensitized with protoporphyrin and suggest that colloid osmotic hemolysis follows damage to the plasma membrane. Concepts reviewed above suggest that the spectral dependence of photohemolysis in normal red cells means that at least two target chromophores are involved: (1) the one associated with non-oxygen dependent hemolysis has an action spectrum less than 300 nm and probably consists of tyrosine and tryptophan residues within membrane proteins. (2) The second chromophore is oxygen dependent with an action spectrum in the 400 nm range which may be due (in normal cells) to small amounts of protoporphyrin IX or to riboflavin.

This second mode of hemolysis may serve as a model for phototoxic damage to membranes by most photosensitizing agents. Without entering a detailed discussion of molecular orbitals, it can be stated that a photon of the appropriate wavelength can promote one of the drug residue's delocalized ring electrons to the first excited electronic state, called a singlet state. Where favorable electric and magnetic environmental conditions allow, some of these excited molecules do not immediately revert to ground state again but may enter a relatively longlived excited condition called the triplet state, whose importance to this discussion lies in its remaining in existence for from 10^{-3} sec up to a full second - long enough for chemical reactions to occur. The presence of an excited drug molecule opens a pandora's box of possible oxidative mechanisms (97).

The favored target or substrate in the above sequence may vary with subcellular localization of drug and other factors, but with several agents histidine residues react more rapidly than tyrosine, tryptophan, cystine or methionine. In DNA most photodynamic drugs preferentially attack guanine. The photoproducts of these attacks have not been identified, but peptide and sugar-phosphate bonds seem to be immune from this form of photodynamic attack.

Carbon-carbon double bonds in unsaturated fatty acids appear to be the most susceptible of cellular lipids, and Goldstein (96) has recently published new evidence for progressive lipid peroxidation in membranes of red cells sensitized with protoporphyrin. Membrane sulfhydryl groups are lost and acetylcholinesterase activity falls before hemolysis takes place, illustrating damage to membrane proteins as well as lipids.

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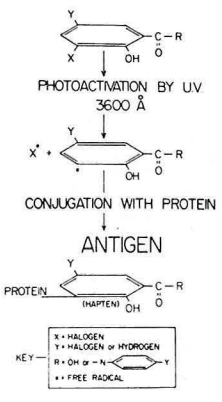
Photoallergic Drug Reactions: Advances during the past decade have rendered these puzzling clinical problems understandable in terms of cell-mediated immune reactions. Other modes of immunologic injury have not yet been detected in this setting.

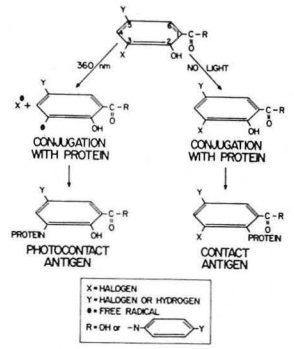
The role of light appears to relate solely to a photochemical reaction which structurally alters the haptenic group or alters its avidity for the carrier protein, which then forms the complete photoantigen as suggested in the following diagram. This mechanism differs from that proposed for cell mediated immune reactions in ordinary contact dermatitis only by the intervention of long wave uv-light.

Major groups of photoallergenic agents in man

Antibacterial compounds
Halogenated salicylanilides and related compounds
Antibacterials
Sulfonamides
Antifungals
a) Griseofulvin
b) Fentichlor
c) Multifungin
d) Jadit
Antihistamines
Promethazine (phenergan)
Blankophores
Cyclamates
Diuretics
Thiazides
Oral hypoglycemic agents
a) Tolbutamide (orinase)
b) Chlorpropamide (diabinese)
Sunscreens
Substituted benzoic acids
Tranquilizers
a) Chlorpromazine (thorazine)
b) Chlordiazepoxide (librium)

The table indicates that, like phototoxic compounds, photoallergenic ones are resonating structures of low molecular weight, most of which fluoresce.





Hypothesis of mechanisms of allergic contact and allergic photocontact dermatitis.

The list is a long one, with halogenated antiseptic compounds used in soaps, cosmetics, and other consumer products justifiably at the top of the list. Careful quantitative studies (98) have shown that under conditions of normal use of these products, sensitive individuals, even those of dark skinned racial background, become vulnerable to painful erythema after less than 5 minutes spent in Texas noonday sun (control caucasians minimal erythema time - 20 minutes).

REFERENCE

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Characteristics of drug-induced photosensitivity (modified from 90)

Reaction		Phototoxic	Photoallergic
Incidence		+++ (could reach 100%)	+
Time Course:	— reaction possible on 1st exposure	+	0
	— incubation period required	0	+
	— time: intensity relationship	single attack	relapses
Characteristics of Response:	— clinical appearance	sunburn-like	varied
	— flares at distant or covered sites	0	+
Antigen Dependence:	— sensitizer chemically altered to produce reaction	0	+, covalent binding
	— crossreactions to structurally related compounds	rare	frequent
	— concentration of antigen needed	high	low
Immunologic Tests:	— passive transfer, MIF	0	+
Action Spectrum		resembles absorption curve	higher than absorption curve

Persistant Light Reactivity: Many unsolved problems remain. Most patients who experience photoallergic contact dermatitis clear promptly with avoidance of antigen and light, but a small minority experience continued outbreaks whenever exposed to light even though they shun contact with the photoallergen. These patients have been called persistant light reactors (99). They may become seriously disabled, since they cannot tolerate even brief exposures to sun, daylight from the sky, or even some sources of artificial light. Their pathologic sensitivity extends over a much broader range than that of any other disorder; they are abnormally sensitive

to the sunburn spectrum and also react to longwave uv and visible light. Their range of sensitivity is thus much broader than the action spectrum of the photosensitizer which originally triggered their reaction.

Occasionally changes resembling cutaneous lymphoma develop in severely affected patients with photocontact dermatitis if they experience continued exposure to the antigen and light (100). It is not clear to what extent the phenomenon of non-antigen-exposed persistent light reactivity may also contribute to this syndrome. The course of one patient seen here at Parkland is worth summarizing with this pattern in mind.

CASE SUMMARY

T.J. a 68 yo Black male retired Pullman conductor came to the Dermatology Clinic in February 1970 complaining of itching and thickening of the skin since 1956. At the time he worked on trains traveling between Texas and California, and enjoyed taking sunbaths during stopovers. In retrospect he recalled using a number of antibacterial or medicinal soaps, one of them a brand known to contain bithionol, a potent photo contact sensitizer.

By 1960 his exposed areas were swollen, cracked, oozing, and painful. He entered a local hospital where biopsies of skin and enlarged lymph nodes were diagnosed as mycosis fungoides. He was described at the time as having a grossly nodular, infiltrated skin, leonine facies, marked hyperpigmentation and hyperkeratosis. Cytoxan was given, and continued until 1971.

On examining him it was apparent that most of the changes were limited to exposed areas. Patch tests to photo-antigens were applied in duplicate. After 24 hours under occlusive tape, one set was exposed to long wave ultraviolet light, the other set protected. On his return two days later severely swollen and blistered rectangular areas were noted beneath bithionol and tribrom salicylanilide on the light-exposed side. The covered side remained negative but a generalized flare of oozing, crusting, active dermatitis developed. A diagnosis of photocontact dermatitis was made and use of medicated soaps (which he had continued to use) was forbidden. Next, the slides from the original skin biopsy sections were compared with a fresh biopsy. While both sections revealed intense inflammation and edema, none of the definitive microscopic criteria for mycosis fungoides could be detected.

Subsequently he has continued to improve on topical steroids and sunscreens and is now nearly free of symptoms.

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DISEASES MARKED BY DEFECTS IN REPAIR OF DNA

Xeroderma Pigmentosum: This is a rare autosomal recessive disorder marked by severe actinic damage beginning before age three, the patients going on to develop many types of cutaneous neoplasia during the teens or even before, and, in most patients, premature death in the teens or twenties from metastases. One form of the disease shows only skin symptoms while an even rarer form displays additional neurological disorders (microcephaly, mental retardation) dwarfism, and hypogonadism. This latter variant is called the de Sanctis-Cacchione syndrome.

Clinically, the very young children with this disease display dark irregular freckles, disproportionate skin dryness, progressive telangiectasia, white atrophic areas, and suffer from slow-healing ulcers that may deform eyelid or nose. Basal cell carcinomas develop most often, but they also provide a field for squamous cell carcinoma and melanoma, the latter a frequent cause of death.

The disease has been recognized for nearly a century. Molecular biologists became interested in it as early as the early 60's when Gartler showed that the patients cultured fibroblasts failed to survive if irradiated with uv (101). But credit belongs to Cleaver for the discovery (102) of a fundamental defect in DNA repair in this disease, the deletion of a mechanism earlier described in bacteria.

Several points should be reemphasized before going further: (1) Normal cells absorbing excessive uv do not die because of damage to DNA. Injury to protein and perhaps lipid is the proximate cause of cell death. Harm has estimated (75) that the germicidal effect of one hour of direct sunlight in Dallas at noon on a clear October day is equivalent to a dose of short uv able to kill 97% of cultured normal human fibroblasts. Yet fibroblasts quite easily repair the damage done their DNA by this dose.

(2) Normal levels of repair of uv-damage in many light-sensitive disease such as the Rothmund -Thomson syndrome show that defects in repair are not the only cause of pathologic photosensitivity.

(3) The association between cancer and failure to repair DNA is an intriquing one but the naive generalizations that attributes all carcinogenesis to lack of repair is false, since numerous human tumor cell lines possess normal repair . A more subtle link must be sought -- perhaps by influence of such tattered DNA on a 3rd process involving mutation.

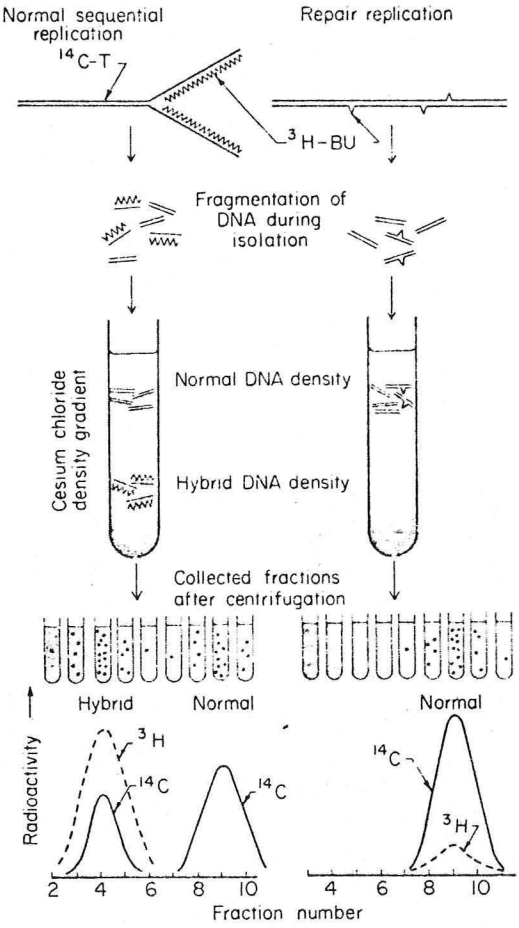
(4) Bacteria seem to possess means of repairing DNA that more complex organisms have lost. Also a certain degree of non-enzymatic recovery can occur without the organism having to provide for it, as noted in the table.

Reversal of Photo Injury (modified from Ref. 97 pp 138-156)

Mechanism	Products Affected	Conditions	Significance In Vivo
Nonenzymatic			
spontaneous decay	hydrate of cytidilic acid	T 1/2 48 min pH 5-7 25°C	+
direct photoreversal	thymine dimers	max at 240 nm (dimerization max at 280 nm)	0
Enzyme-catalysed			
light- or photo-dependent	pyrimidine dimers (T-T most rapid)'	binds to irrad. DNA in dark, regenerates in light. (360-390 nms)	+++ widespread in bacteria plants, but animals only up to marsupials
Dark- or excision-repair	pyrimidine dimers	binds, excises, i.e. inserts new pyrimidine bases	+++ general: e. coli to mammals
post replication-repair	pyrimidine dimers	joins sister strands to repair gaps remaining opposite dimers after replication	bacteria

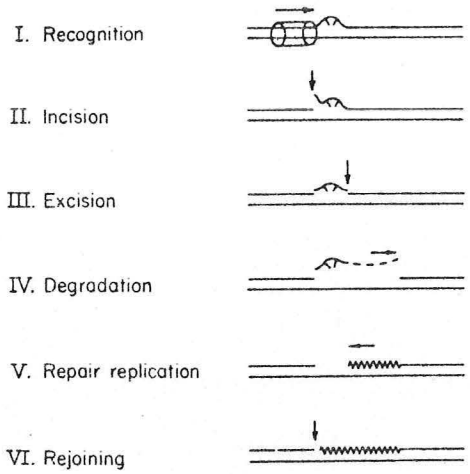
Evidence For Excision-Repair: (Ref. 97, pp 138-156) Evidence of several sorts has lead to the conclusion that normal fibroblasts excise and replace thymine dimers while XP fibroblasts, and by implication all his other cells, cannot. 1) As Gartler showed, XP cells are killed more rapidly than normal. 2) Autoradiography shows that XP cells exhibit very little unscheduled DNA synthesis after irradiation. Normally one sees two sorts of nuclear labelling if one adds a pulse of tritium-labelled thymidine to an irradiated culture and shortly afterward fixes, imbeds and prepares it for autoradiography. A fraction of the cells happen to be replicating in the usual way. These are cells in the S phase of the cell cycle performing scheduled DNA synthesis. Their nuclei show dense clouds of tritium emission in the emulsion corresponding to their relatively rapid incorporation of thymidine. But after uv exposure a few lightly labelled cells are also seen. These are not in S, but are performing unscheduled DNA synthesis. They are excising and replacing thymine dimers induced by uv damage. In XP cell cultures after irradiation one sees few or no cells in unscheduled DNA synthesis. 3) Density gradient methods. Direct physical evidence for a patch mechanism was provided by Pettijohn and Hanawalt (103) who adapted the density labelling technique first used to prove that DNA replicates by making one new strand on one old one. They used a heavy analogue of thymine, 5 bromo -uracil or 5-BU, tagged with tritium so they could locate it easily. Normal cultures undergoing scheduled DNA synthesis produced centrifugal patterns in which the tritium label all followed the heavier, 5-BU containing, hybrid DNA.

RECONSTRUCTION OF DAMAGED DNA



Irradiated normal cultures however, produced patterns in which label was found not only at the expected hybrid location, but also at the location of the parent DNA strands (in the right side of the figure normal replication has been blocked pharmacologically to keep things neat) meaning that these strands have incorporated very few labelled 5-BU residues, enough to detect the radiolabel but not enough to affect density. XP cells show no label in the parent location, meaning they cannot cut and patch.

Subsequent studies have identified three or four enzymatic steps needed to perform repair - an endonuclease is needed to recognize the dimer and make a nick in one chain, excision and replacement of a variable length of affected strand may be carried out as separate or as concurrent steps, and a ligase is needed to link up the ends again. It is the endonuclease which XP patients lack. They can repair x-ray injury in which particles of sufficient energy to cause chain breakage is used and where the nick is made for them, but not uv injury (or similar injury) in which a chemical bump is formed.



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The deSanctis-Cacchione syndrome: An ingenious experiment utilizing cell fusion has recently shown that XP cells and deSanctis-Cacchione cells complement each other. That is, fused cells bearing both sets of genetic information seem to carry out excisional repair of uv-damaged DNA in a normal fasion. The simplest interpretation of this experiment is that a different gene and enzyme are at fault in the deSanctis-cacchione disorder from that in XP. But occasionally different allelic expressions at the same gene locus can complement in this way, so further studies are required before one can be sure.

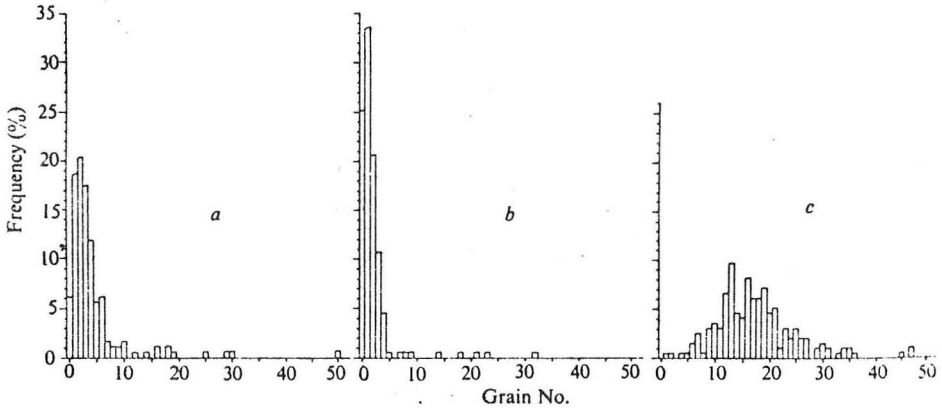

Fig. 3 Frequency distribution of grain counts made of the three types of binuclear cells after ultraviolet exposure that can be identified by atebrin staining of XP4/XP12 fusions. *a*, Binuclear cells having two nuclei with a Barr body (XP4/XP4); *b*, binuclear cells having two nuclei with Y chromatin (XP12/XP12); *c*, binuclear cells having one nucleus with a Barr body and the other nucleus with Y chromatin (XP4/XP12).

NOTE

XP4 cells were derived from a female with ordinary XP.
XP12 cells were derived from a male with the deSanctis-Cacchione syndrome.
The last, or c distribution, is identical to that shown by normal cells.

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PROTOCOL FOR EVALUATION OF PHOTOSENSITIVE DISEASES

Day 1: History and physical exam: Careful evaluation of age of onset, family history, associated diseases, (alcoholism, symptoms of connective tissue disease), medications, contact with plants or perfumes, with agents known to contain photocontact sensitizers.

Laboratory: Routine studies plus antinuclear factor, skin biopsy for H and E and immunofluorescent band test.

Porphyrin screening tests for office use (105): Blood: to 5 ml of a 5:1 mixture of ether:acetic acid add 4 drops of blood and mix thoroughly with glass rod, decant to second tube, add 1 ml 3N HCl and mix well. Let stand for 10 minutes. Examine with black light. Red fluorescence in lower layer suggests protoporphyria. Urine: to 4 ml urine add 10 drops acetic acid and 1 ml amyl alcohol. Shake gently, transfer upper alcohol layer to a second tube and shake with 10 ml 1.5N HCl. Examine with black light. Pink red fluorescence indicates uro- or coproporphyrinuria. (P.C.T. or variegate) (Fresh urine may occasionally yield a false negative due to porphyrinogens. Add .05% iodine and repeat test)

Day 2: Determine minimal erythema dose using solar-quality uv lamp. Apply duplicate patch tests to common photocontact allergens (106).

Day 3: Record MED readings. Remove one set of patches and give 40 x MED through window glass filter. Expose one unfiltered site to 10 x MED, repeat this last step weekly or biweekly x5. Biopsy if a lesion resembling a photoallergic one develops.

Day 5: Read light-exposed and light-shielded patch test sites at 48h (repeat at 96h - some reactions are delayed)

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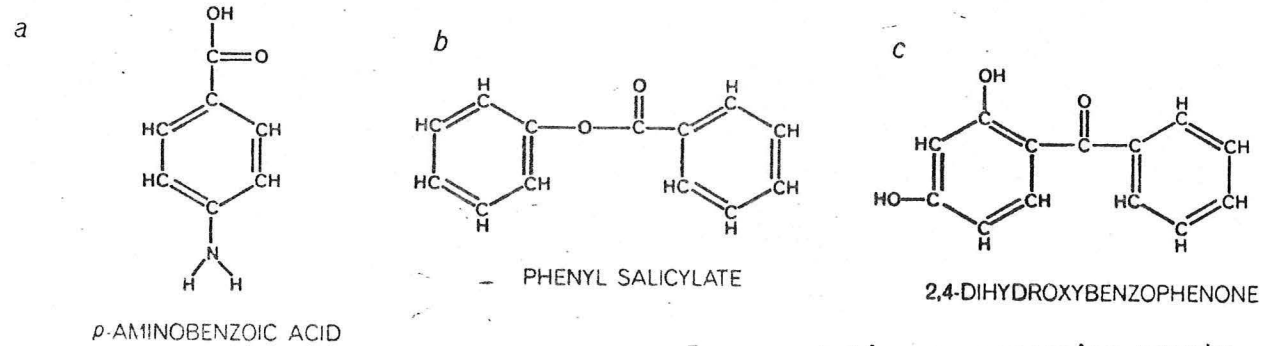
PROTECTION OF SKIN AGAINST LIGHT

Topical preparations: The requirements for an ideal topically applied filter are not the same for all clinical situations. The normal individual preparing for a day at the beach needs protection only in the 290-320 nm range. The porphyric and the drug-photosensitized patient need protection from much longer uv and visible. The persistant light reactor may need interdiction of all but very red light, and the surgeon or bacteriologist exposed to the 254 nm mercury line of sterilizing lamps may need protection against very short uv. Add to these requirements the need for convenient application, persistance through sweating and swimming, and acceptable appearance and the formidable nature of the challenge is apparent.



Representative sunscreening agents

Use of Topical Photoprotective Agents

Setting	Agent	Comment
Normal persons (erythemogenic spectrum)	5% paraminobenzoic acid (107) Tradenames: Pabanol(Elder) Presun(Westwood)	stains on white clothing wash out easily. dries clear, some resistance to sweat elution.
Porphyria, drug (long UV)	benzophenone derivatives (108) Tradenames: Solbar(Person & Covey) Uval(Dome)	lotion base, little resistance to sweat elution
	DHA-Lawsone (experimental 109)	binds to and pigments stratum corneum with good resistance to elution. somewhat less effective.
Persistant light reactor (UV and visible)	opaque agents-zinc oxide titanium dioxide Tradenames: RV Paque(Elder)	heavy clothing and/or avoidance more practical.
Germicidal exposure (254 nm)	benzophenone derivatives	

Systemic preparations: Psoralens. Psoralens plus light exposure induce both dense tanning and a thickened, compact, stratum corneum. Taken by persons with vitiligo they may lead to worthwhile repigmentation. But a knowledge of their mode of action inspires distrust with regard to later carcinogenic effects, and their casual use to promote suntan should be discouraged. Two preliminary studies failed to document protection against recurrent skin cancer (quoted in 6).

4-aminoquinolines. Chloroquin and its congeners limit damage from photosensitivity reactions, but have no effect on normal UV-erythema. It, along with other agents that ameliorate rheumatoid arthritis as well as photosensitivity,has been thought to act by inhibiting release of lysosomal hydrolases from inflammatory cells (110). Retinal toxicity limits its use, although careful perimetry with red targets may make the diagnosis of premaculopathy possible at a reversible stage (111).

Carotene. In a classic example of undirected research bearing fruit, Mathews-Roth has developed a new agent for safe systemic protection against visible and long UV light (112). She was led to the use of this agent by finding that certain nonpigmented bacteria were easily killed by photosensitizing dyes plus light. Adding back the wild type carotenoid pigment let them survive. Patients with disabling and previously untreatable erythrohepatic protoporphyria have experienced striking increases in tolerance to light after only 60 mg a day of the active principle. The persistent light reactor and others sensitive

to visible light should also respond. Patients with LE or XP will derive little benefit from carotene, however, since their sensitivity lies in the sunburn range.

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