4. Other Relevant Data

4.1 Transmission and absorption in biological tissues

UVR may be transmitted, reflected, scattered or absorbed by chromophores in any layer of tissue, such as the skin and eye. Absorption is strongly related to wavelength, as it depends on the properties of the responsible chromophore(s). Accordingly, transmission is also wavelength-dependent. Transmission and other optical properties are affected by changes in the structure of the tissue and, especially in the case of the lens of the eye, by ageing.

Absorption of radiation by a tissue chromophore is a prerequisite for any photochemical or photobiological effect; however, absorption does not necessarily have a biological consequence.

4.1.1 *Epidermis*

Since UVR-induced skin cancer is an epidermal phenomenon, this section focuses on epidermis and excludes the dermis.

The epidermis, a tissue with a high replication rate, can be divided functionally into two: an inner, living part (60–160-μm thick in humans) of cells at various stages of differentiation and the outermost, non-living, terminally differentiated stratum corneum (8–15-μm thick in humans). The dividing cell population is located in the innermost basal layer of the living epidermis. Optical properties have usually been studied using isolated strateum corneum or whole epidermis. Absorption and scattering of UVR by the stratum corneum afford some protection to the living part of the epidermis from UVR exposure.

Human and mouse epidermis have important structural differences. The living part and the stratum corneum of human epidermis have about 10 cell layers each. In mice, the living part has two to three cell layers and the stratum cornea one to two cell layers. The interphase of human epidermis and dermis is highly undulated (i.e., epidermal thickness varies), whereas in the mouse it is flat.

Skin contains sebaceous glands which secrete lipid-containing sebum, which forms a film on the stratum corneum.

(*a*) *Humans*

The optical properties of human skin have been reviewed (Anderson & Parrish, 1981, 1982).

Everett *et al.* (1966) used a variety of methods to obtain whole epidermal and stratum corneum preparations of human skin. Transmission characteristics (from 240 to 700 nm) were measured using a recording spectrophotometer *via* an integrating sphere which permits the measurement of forward scattered radiation. Transmission values of whole epidermis in

white skin ranged from 1% at 250 nm to 44% at 320 nm, while transmission at 400 nm was about 50%.

Kaidbey *et al.* (1979) compared the optical properties (250–400 nm) of whole epidermis and stratum corneum from black and white skins. In general, the absorption spectra from the stratum corneum were similar in shape and magnitude; however, the absorption spectra for whole epidermis were clearly different: At about 300 nm, the absorbance (accounting for scattering) of black epidermis was twice that of white epidermis.

Anderson and Parrish (1981, 1982) presented data which show that epidermal transmission between 260 and 290 nm will be overestimated if no correction is made for tissue fluorescence (330–360 nm). This is most evident at about 280 nm and is consistent with tryptophan or tyrosine fluorescence.

Bruls *et al.* (1984a) measured transmission in whole human epidermis and stratum corneum of UVR between 248 and 546 nm, using a solar blind detector which corrects for fluorescence, and found results different from those of Everett, in particular, that UVC transmission was one to two magnitudes lower. The transmission spectra of whole epidermis and stratum corneum showed a similar general shape but with differences in minima and magnitude. The minimum for epidermis was 265 nm and that for stratum corneum was 275 nm, presumably reflecting different chromophores in those tissues. At 254 nm, transmission in stratum corneum was about two orders of magnitude greater than that in whole epidermis. At about 300 nm, this difference was only one order of magnitude. The transmission in stratum corneum from previously sun-exposed skin was about one order of magnitude less than that in unexposed epidermis at 254 nm. The difference was less at wavelengths > 290 nm. The minimal transmission in stratum corneum from previously sun-exposed skin was shifted from 275 to 265 nm. The authors also showed that the relationship between tissue thickness and transmission of UVR and visible light (log scale) is linear.

Bruls *et al.* (1984b) studied the relationship between the MED of UVB (filtered mercury arc) and UVC (germicidal lamp) and epidermal transmission. A clear linear (log–log) relationship was demonstrated; the MED increased with decreased transmission. Repeated exposure to UVB resulted in higher MEDs of UVB and UVC and decreased transmission of UVB (only epidermis measured) and UVC (epidermis and stratum corneum measured).

Beadle and Burton (1981) extracted skin lipids from human scalps and measured their transmission spectra in hexane. They estimated that lipid concentrations normally present on the skin surface of the forehead would reduce transmission at 300 nm by about 10%.

(*b*) *Experimental systems*

No data are available on transmission in the stratum corneum of mice. Sterenborg and van der Leun (1988) measured transmission of 246–365 nm in Skh-hr 1 mouse epidermis *in vitro*. Minimal transmission (about 2%) was observed at 254 nm and 270 nm; 10% was transmitted at 290 nm, 50% at 313 nm and 70% at 365 nm. Agin *et al.* (1981a) studied changes in optical properties of the epidermis of six to eight Skh-1 albino and Skh-2 pigmented (ears and tails) hairless mice irradiated dorsally with a single, 125-h exposure to a UVA source (GE F8T5-BL) with and without a 3-mm glass filter. When unfiltered, 1.4% of the radiation was < 320 nm and when filtered, 0.12% was < 320 nm. The mid-back (whole epidermis) was examined by forward scattering absorption spectroscopy (250–400 nm) at

48 h, 96 h, nine days and 23 days. With the filtered source, there was an increase in absorbance across the spectrum at 48 h, and the absorption spectrum was similar to that of control skin. Transmission returned to the control baseline by 23 days. With the unfiltered source, there was a smaller increase towards baseline absorbance at 48 h. With time, there was a general decrease in absorbance, except at 250–280 nm at which there was an increase at nine and 23 days. At 23 days, the spectrum had not returned to baseline level, despite a normal histological appearance.

de Gruijl and van der Leun (1982a) studied the effect of repeated exposure to UVR on epidermal transmission in Skh-hr 1 hairless albino mice. Groups of 11–40 mice were exposed to daily doses of UVR ranging from 0.11 to 1.9 kJ/m^2 from Westinghouse FS-40 sunlamps. Transmission measurements corrected for fluorescence of the epidermis were made at 313, 302 and 297 nm. After six weeks' exposure, the higher daily doses resulted in decreased transmission at all wavelengths. The optical density (the negative logarithm of transmission) ratios for the three wavelengths were fairly constant with each dose. There was a simple linear relationship between duration of treatment, increased optical density at 297 nm and epidermal thickness, measured microscopically from frozen sections, which indicates that increased optical density is a result of UVR-induced epidermal hyperplasia. These data show that UVR-induced changes in epidermal transmission may modify the UVR dose–response relationship for skin cancer.

(c) *Epidermal chromophores*

The influence of chromophores on the optical properties of the epidermis has been reviewed by Anderson and Parrish (1981). The main chromophores are urocanic acid (λ_{max}, 277 nm at pH 4.5), DNA (λ_{max}, 260 nm at pH 4.5), the aromatic amino acids tryptophan (λ_{max}, 280 nm at pH 7) and tyrosine (λ_{max}, 275 nm at pH 7), and melanins (Morrison, 1985).

Urocanic acid is the deamination product of histidine and is present in human and guinea-pig epidermis (mainly stratum corneum) at about 35 μg/cm^2 dry weight. It exists in two isomers, *trans* (E) and *cis* (Z); the *trans*-isomer is converted to the *cis*-isomer upon UV irradiation. The absorption spectra of the two isomers are virtually superimposable, but the extinction coefficient of the *cis* isomer at λ_{max} is 20% lower (Morrison, 1985). Norval *et al.* (1988) quantified urocanic acid isomers in mouse (C3Hf Bu/Kam) skin during development and after exposure to UVB radiation. Fetal dorsal mouse skin had a low total urocanic acid content, which increased in neonatal and older animals. Exposure to UVR increased the proportion of the *cis*-isomer within 16 h from 4.7% in nonirradiated mice to 31%, and this was maintained for days (16% after seven days). The photostationary state for in-vivo isomerization in guinea-pig skin is 45% *cis*-/55% *trans*-isomer (Baden & Pathak, 1967).

DNA is not present to any extent in the stratum corneum of guinea-pigs (Suzuki *et al.*, 1977). Bruls *et al.* (1984a) attributed the differences in transmission minima between whole epidermis (265 nm) and stratum corneum (275 nm) in humans to the lack of DNA. Absorption by protein occurs throughout the epidermis.

Melanins are stable protein polymers packaged in melanosomes, produced by melanocytes and transferred to keratinocytes. Melanins absorb broadly over the UV and visible spectrum although they are not neutral density filters of the skin. For example, 3,4-dihydroxyphenylalanine (dopa)–melanin shows a steady decline in optical density

between 210 and 340 nm (Anderson & Parrish, 1981). There is no significant racial difference in the number of melanocytes/unit area of a given body site (Szabó *et al.*, 1972), so that differences in the transmission properties of black and white skin are believed to be due to differences in melanin content and in the packaging and distribution of melanosomes in the epidermis (Kaidbey *et al.*, 1979).

(*d*) *Enhancement of epidermal penetration of ultraviolet radiation*

Prolonged exposure of skin to water increases sensitivity to UVB. This effect is thought to be due to the removal of UVR-absorbing compounds, especially urocanic acid, from the stratum corneum (Anderson & Parrish, 1981).

Spectral remittance at 300–400 nm has been measured in normal and psoriatic white skin after the application of mineral oil. No effect was observed in normal skin, but remittance in psoriatic skin was reduced within seconds after application of oil, implying greater transmission (Anderson & Parrish, 1982). A similar enhancement of transmission was proposed to explain the observation that topically applied arachis oil enhances tumorigenesis by solar-simulated radiation in hairless albino mouse skin (Gibbs *et al.*, 1985).

4.1.2 *Eye*

(*a*) *Humans*

Boettner and Wolter (1962) measured transmission of direct and forward scattering UVR (220–400 nm) in the cornea, aqueous humour, lens and vitreous humour from nine freshly enucleated normal eyes. There was no corneal transmission of < 300 nm, beyond which the transmission spectrum showed a very steep increase to about 80% transmission at 380 nm (the curve was almost vertical between 300 and 320 nm). Aqueous humour transmitted > 220 nm, with a steep rise to 90% transmission at 400 nm and no evidence of scattering. In a young (4.5-year-old) lens, transmission started at 300 nm with a peak at 320 nm, declining sharply to no measurable transmission between 370 and 390 nm; thereafter, it showed a steep increase. A similar but slower pattern was reported for two older lenses (53 and 75 years old), with greater light scattering. Transmission in the vitreous humour began at 300 nm with a steep increase to 80% transmission at 350 nm. Lerman (1988) showed that transmission of UV at 300–400 nm in normal human lenses decreases with age between three days and 82 years. A review by Sliney (1986) stated that 1% of incident radiant energy in the 300–315 nm range reaches the human retina early in life.

(*b*) *Experimental systems*

Kinsey (1948) measured transmission of direct UVR [no mention of instrumentation to detect scattering] in the corneal epithelium, whole cornea, aqueous humour, lens and vitreous humour of young adult albino rabbits. The cornea, aqueous and vitreous humor absorbed virtually all radiation at < 300 nm; the lens absorbed > 90% radiation at wavelengths < 370 nm.

Bachem (1956) measured absorption of UVR at 293–435 nm by the lens and cornea from rabbit eyes. Few technical details were given, but the author indicated that scattering was taken into account. The cornea absorbed all radiation at 293 nm, and the lens absorbed

all radiation < 334 nm. Calculation of absorption by the lens *in situ* gave a maximum at 365 nm, with little or no absorption at > 400 and < 300 nm.

Ringvold (1980) studied the absorption of UVR at 200–330 nm by cornea from young adult albino rabbits, rats, guinea-pigs and domestic cats. In contrast to the results of other studies, the cornea did not completely absorb wavelengths < 300 nm; depending on the species, absorption at 300 nm ranged from about 30 to 80%. [The Working Group noted that this discrepancy cannot be explained by scattering, as presumed failure to take its effect into account would overestimate absorption.]

4.2 Adverse effects (other than cancer)

This section deals generally with adverse effects of UVR; however, beneficial effects also occur in humans. The vitamin D_3 precursor, previtamin D_3, is formed in the epidermis and dermis through the photochemical action of UVB (Holick *et al.*, 1980). The total daily requirement of vitamin D_3 (cholecalciferol) is supplied in most people by the combination of synthesis in the skin and contribution from dietary sources of animal origin. Older people are at particular risk for developing vitamin D_3 deficiency, partly because the capacity for its formation decreases with age (MacLaughlin & Holick, 1985). The sunscreen *para*-amino-benzoic acid efficiently blocks the photosynthesis of previtamin D_3 in the skin (Matsuoka *et al.*, 1987). It has been estimated that exposure of the cheeks for 10–15 min in the midday sun in Boston, USA, would be sufficient to provide the daily requirement of vitamin D.

4.2.1 *Epidermis*

(*a*) *Humans*

The most prominent acute effects of UVR on human skin are erythema ('sunburn') and pigmentation, with cellular and histological changes.

(i) *Erythema and pigmentation (sunburn and suntanning)*

Dose–response curves for erythema were constructed for four radiation wavelengths, 254, 280, 300 and 313 nm, by Farr and Diffey (1985); the erythemal response on the back was assessed quantitatively by a reflectance instrument. At 254 nm, erythema was maximal approximately 12 h after irradiation at doses up to about five times the MED. At higher doses, erythema was more persistent, with little change in intensity from about 12 h to at least 48 h after irradiation.

At 313 nm, with doses around the MED, the maximal response was seen 7 h after irradiation; with doses of two to three times the MED, the maximal response occurred at about 4 h. The MED at 254 and 280 nm was substantially lower than that at 300 and 313 nm; however, the slopes of the dose–response curves for erythema with 254 nm and 280 nm radiation were much flatter than those at 300 nm and 313 nm (Farr & Diffey, 1985).

The time-course of UVA erythema following irradiation with a high-intensity UVA source (predominantly 360–400 nm) was found to be biphasic. Erythema, which may be due to heat, was present immediately. It was minimal at about 4 h then increased between 6 and 24 h. The intensity of the early phase was dose-rate dependent, whereas the intensity in the latter phase depended on dose only. The slope of the log dose–erythema response to UVA at 24 h did not differ from that to UVB (Diffey *et al.*, 1987).

A number of variables affect the observation of erythema, including anatomical site, time of observation after irradiation, size of irradiated area, method of recording erythema and season (Diffey, 1982).

The pharmacological changes that may be responsible for erythema have been studied. Plummer *et al.* (1977) examined suction blisters raised on UVB-inflamed human abdominal skin. Bioassayable prostaglandin activity was elevated 6 and 24 h after irradiation, and levels of prostaglandin $F_{2\alpha}$, measured by radioimmunoassay, were elevated at 24 h; levels had returned to normal at 48 h, but erythema persisted. Greaves *et al.* (1978) extended these observations. Following UVC irradiation, arachidonic acid and prostaglandin E_2 and F_2 levels were elevated at 6 h, reached a maximum between 18 and 24 h, when erythema was most intense, but returned to control levels by 48 h, at which time the erythema had subsided. Indomethacin substantially reduced blood flow, with a good correlation between the reduction in visible erythema and prostaglandin E_2 and F_2 activity in irradiated skin. The results are compatible with the view that UVC-induced erythema is mediated by products of arachidonic acid metabolism. Changes in UVB-induced erythema were similar to those with UVC at 24 h, but by 48 h the levels of arachidonic acid and of metabolites had returned to normal, although erythema persisted. Further, although indomethacin suppressed prostaglandin formation, it altered blood flow only slightly, indicating that other factors must play an important role in inflammation following UVB irradiation. Elevated histamine levels have also been observed, but antihistamines have little effect in diminishing erythema (Gilchrest *et al.*, 1981).

Increased pigmentation of the skin by UVR occurs in two distinct phases: immediate pigmentation and delayed tanning (Hawk & Parrish, 1982; Gange, 1987). Immediate pigmentation, thought to result from oxidation and redistribution of melanin in the skin, begins during irradiation and is maximal immediately afterwards; it occurs following exposure to UVA and visible light and may fade within minutes or, after greater doses to people with darker skin, may last up to several days. Delayed tanning is induced maximally by exposure to UVB and becomes visible about 72 h after irradiation. It is associated with an increase in the number of melanocytes as well as with increased melanocytic activity, elongated dendrites, increased tyrosinase activity and increased transfer of melanosomes to keratinocytes. Small freckles may be formed, particularly in fair-skinned individuals.

Not all pigmentary changes induced by UVR are localized at the site of irradiation. Experimental exposures to UVB three times a week for eight exposures at the MED increased the number of melanocytes and produced larger, more dendritic melanocytes in both exposed skin and, to a much lesser extent, areas of skin shielded from the radiation. The increase in melanocyte number in both exposed and covered areas was greater in individuals whose melanocyte density was lower prior to exposure than in individuals with a high initial density (Stierner *et al.*, 1989).

The erythemal and tanning responses of human skin are genetically determined. Responses to a first seasonal exposure of about 30 min to the midday sun have been used as part of the basis for a skin type classification for white-skinned people ranging from Celtic to Mediterranean (Morison, 1983a; Pathak *et al.*, 1987):

Skin type I Always burn, never tan
Skin type II Usually burn, tan less than average (with difficulty)

Skin type III Sometimes mild burn, tan about average
Skin type IV Rarely burn, tan more than average (with ease)

UVA radiation produces immediate changes in melanocytes in white-skinned people. In individuals with type-II skin, multiple pinocytotic vesicles, larger vacuoles, swelling and partial-to-total dissolution of the inner membranes of mitochondria and numerous small vesicles associated with an enlarged Golgi apparatus were seen with doses that did not produce immediate pigment darkening (Beitner & Wennersten, 1983). In those with type-III skin, similar changes occurred but only with doses that produced immediate pigment darkening (Beitner, 1986).

Three Japanese skin types have been described on the basis of personal reactions to the sun (Kawada, 1986). Experimental exposure to monochromatic UVR showed that the MED correlated well with skin type. Immediate tanning occurred but was not related to skin type. After irradiation with the minimal dose that would produce immediate tanning, the tan faded within 3–15 min; after greater exposures, the tan remained longer but never for more than 60 min. The action spectrum for immediate tanning had a maximum at 320 nm and decreased gradually towards 400 nm. New pigment formation (delayed tanning) after exposure to 290 nm and 305 nm radiation began about 65 h after irradiation and increased until it reached a maximum at 124 h (with a dose four times the MED) or 151 h (with a dose eight times the MED). Following a dose three times the MED, some delayed tanning was still evident after two months. The minimal melanogenic dose (producing delayed tanning) was greater than the MED for all Japanese skin types, in contrast to findings in white Caucasians.

Parrish *et al.* (1981) showed that repeated daily exposure to doses of broad-band UVB and UVA lower than the MED lowered the threshold for both erythema and true melanogenesis for several subsequent days; the threshold for melanogenesis was decreased to a greater extent than that for erythema, a separation that was more pronounced for UVA than for UVB radiation.

(ii) *Pigmented naevi*

Exposure to the sun appears to stimulate the occurrence and behaviour of acquired pigmented naevi. Kopf *et al.* (1985) showed, in 80 consecutive patients with dysplastic naevus syndrome, that the concentration of naevi on areas of the thorax protected relatively well from the sun was substantially lower than that on areas exposed to the sun. Augustsson *et al.* (1990) showed that, in melanoma cases as well as in controls, the concentration of common naevi was higher on the sun-exposed skin of the back than on the protected skin of the buttocks. An Australian study compared naevi excised in summer to those excised in winter in Western Australia. Inflammation, regression, mitotic activity and lymphocytic infiltration were significantly more prevalent in naevi excised in summer than in winter (Holman *et al.*, 1983b; Armstrong *et al.*, 1984). [The Working Group noted that these observations may be confounded by the site of the naevi.]

In an Australian cross-sectional study of 511 people, the presence of palpable naevi on the forearm was associated with female sex, young age, not having southern European grandparents, being born in Australia and intermediate categories of variables indicating sun exposure (Armstrong *et al.*, 1986).

Gallagher *et al.* (1990a,b) studied risk factors for common naevi in school children in Vancouver, British Columbia, Canada. The number of naevi increased with age (from six to 18 years). Naevi occurred most commonly on intermittently than on constantly exposed parts of the body and less commonly in skin that was rarely exposed. Light and freckled skin, propensity to burn rather than tan upon exposure to the sun and a history of frequent or severe sunburn were associated with a large number of naevi.

Green *et al.* (1988b) compared the prevalence of melanocytic naevi (benign pigmented moles) in children aged 8–9 in Kiddermister, United Kingdom, and Brisbane, Australia. Regardless of skin colour, the mean number of naevi was at least five times larger in the Australian children than in the British children. In both populations, naevi were more prevalent in children with fair skin.

(iii) *Ultrastructural changes*

Jones, S.K. *et al.* (1987) and Roth *et al.* (1989) each described a patient who developed many freckle-like lesions on all exposed sites following repeated exposure to high-dose UVA from a home sunbed for tanning the skin. Biopsy showed increased numbers of large melanocytes in the basal layers.

Rosario *et al.* (1979) examined the sequential histological changes produced by single exposures to UVA, UVB and UVC radiation on untanned skin of the lower back. Exposures were designed to cause approximately equal degrees of erythema. Following UVB and UVC, dyskeratotic cells ('sunburn cells') were scattered throughout the malpighian layer of the epidermis at 24 and 48 h. By 72 h and seven days, they formed a continuous band in the upper malpighian layer or the stratum corneum. Epidermal hyperkeratosis, parakeratosis and acanthosis appeared concurrently at 72 h. The granular layer was focally absent at 24 and 48 h and had increased focally at 72 h and seven days. There was a minimal-to-moderate lymphocytic infiltrate in the dermis which was most pronounced after 48–72 h. Infrequent mitotic figures were observed in keratinocytes. UVA caused fewer dyskeratotic cells at all time intervals, and these never coalesced into a band. UVA, however, elicited the greatest degree of inflammation at 24, 48 and 72 h in terms of both quantity and depth of cellular infiltrate. Endothelial cell swelling, nuclear dust and extravasation of red blood cells were generally observed together. These dermal findings were more pronounced at 72 h. Neither epidermal hyperkeratosis, parakeratosis nor acanthosis was observed. Intracellular oedema of moderate degree was noted with all wavebands at all time intervals. The authors considered that the production of more prominent dermal changes by UVA than by UVB and UVC might be related to greater penetration of longer wavelengths. The histological changes returned to normal earliest after UVB and latest after UVA irradiation.

Pearse *et al.* (1987) examined the effects of repeated irradiation with UVB (0.5, 1 and 2 times the MED three times a week for six weeks) and UVA (6 J/cm^2 [60 kJ/m^2] three times a week for three weeks). UVB irradiation at twice the MED led to significant increases in epidermal thickness, stratum corneum thickness and keratinocyte height, as did UVA irradiation. Both UVA and UVB significantly increased glucose-6-phosphate dehydrogenase activity and decreased succinic dehydrogenase activity throughout the epidermis. The autoradiographic labelling index was significantly increased following the highest dose of UVB.

The benign skin changes attributed to sunlight and seen on physical examination include wrinkles, atrophy, cutis rhomboidalis nuchae (thick, yellow, furrowed skin, particularly on the back of the neck), yellow papules and plaques on the face, colloid milium (firm, small, yellow, translucent papules on the face, forearms and hands), telangiectasia, diffuse erythema, diffuse brown pigmentation, ecchymoses in sun-damaged areas, freckles, actinic lentigo (large, irregular, brown areas), Favre–Racouchot syndrome (yellow, thick comedones and follicular cysts of the periorbital, malar and nasal areas) and reticulated pigmented poikiloderma (reddish-brown reticulated pigmentation with telangiectasia and atrophy and prominent hair follicles on exposed chest and neck) (Goldberg & Altman, 1984). Although most commonly seen in fair-skinned Caucasians, these changes may also be seen in Chinese heavily exposed to the sun (Giam, 1987). A visual system using facial photographs has been developed to enable grading of the degree of elastosis (Cameron *et al.*, 1988).

Holman *et al.* (1984a,b) made silicone rubber moulds of the microtopography of the skin of the hands of 1216 subjects and developed a grading system to describe alterations in skin surface characteristics observed under a low-power microscope. Using multivariate analysis, independent risk factors for topographic evidence of actinic skin damage were: male sex, age, tendency to burn upon exposure to the sun and outdoor occupation. Similar results were reported by Green (1991).

Everett *et al.* (1970) reported ultrastructural changes in the epidermis of six elderly, fair-skinned, freckled, blue-eyed, Caucasian male farmers with a history of multiple actinic keratoses and skin cancers. Light microscopy showed effacement of epidermal rete ridges and an irregular decrease in epidermal thickness in areas of skin exposed to sunlight. Three groups of changes were apparent upon transmission electron microscopic examination: firstly, local areas of degeneration involving groups of adjacent cells, with degenerative changes resembling dyskeratosis in both the basal and the spinous layers of the epidermis; secondly, disturbed cellular cohesion, with variable numbers, distribution and degrees of maturity; and thirdly, changes in epidermal pigment—with the melanin concentration varying from none to excessive—and melanosome complexes that were often abnormally large.

Kligman (1969) described the changes in elastic tissue (elastic hyperplasia or actinic elastosis) seen in the dermis of sun-exposed Caucasian facial skin. Such changes were quite advanced before the extent of the damage became visible clinically. Some elastic hyperplasia was seen in elderly blacks over the age of 70, but the changes were markedly less extensive than those seen in whites.

Bouissou *et al.* (1988) studied elastic fibres in protected skin and skin highly exposed to the sun from normal Caucasians of different ages, using light and electron microscopy. In skin exposed to the sun, there was elastotic degeneration in the reticular dermis and progressive thickening and curling of the elastic fibres in the upper dermis. Altered fibres progressively formed thick, irregular masses, with clumps of amorphous, granular, elastotic material and large areas of uneven staining appearing frequently thereafter. Electron microscopy revealed that normal collagen and elastotic material were often contiguous but never continuous.

(iv) *Keratosis*

The occurrence of keratosis, a benign but probably premalignant squamous neoplasm of the skin (Marks *et al.*, 1988), has been studied in relation to exposure to sunlight in several cross-sectional studies.

Chronic solar damage (assessed by cutaneous microtopographs and paraocular photographs) was associated with keratosis, after adjustment for age, in a study of 1216 people in Busselton, Australia (Holman *et al.*, 1984a). A similar association between cutaneous microtopography and prevalence of keratosis was observed by Green (1991) in a study of 1539 people in Nambour, Australia.

Vitasa *et al.* (1990) conducted a study of 808 white watermen in Maryland, USA. The prevalence of keratosis was 25%. The risk factors for this condition were found in a multivariate analysis to be age, individually estimated cumulative exposure to sunlight, blue eyes, childhood freckling and a tendency to sunburn.

Marks *et al.* (1983) studied 2113 adults in Maryborough, Australia. The prevalence of keratosis was 56.9%. Adjusted for age, the prevalence of keratosis was significantly associated with being born in Australia, with a tendency to sunburn and not tan and with blue eye colour. In another survey by these authors, of 2000 adult in-patients from a hospital in Melbourne, Australia, the prevalence of keratosis on the light-exposed areas of the head and neck, forearms and back of hands was 37.7%. Prevalence of keratosis was significantly associated with age and with being born in Australia and, among men, with outdoor occupation (Goodman *et al.*, 1984). The Melbourne and Maryborough populations were compared further by Marks and Selwood (1985), who attributed the higher prevalence of keratosis in Maryborough to the fact that this population had a 14.2% higher erythemal UVR level.

Foley *et al.* (1986) studied 766 consecutive patients with keratosis. Lesions on the hands and forearms in men were seen more often on the right side than on the left, which the authors attributed to the higher exposure of the right side while driving an automobile. In women, more lesions of the head and neck were on the left side.

(v) *Photosensitivity disorders*

Abnormal reactions to solar radiation, termed photosensitivity disorders, occur in a relatively small number of exposed individuals; these have been reviewed comprehensively (Harber & Bickers, 1981; Bernhard *et al.*, 1987). Genetic and metabolic diseases that may be associated with photosensitivity include xeroderma pigmentosum, phenylketonuria, Bloom's syndrome, Cockayne's syndrome, Rothmund–Thomson syndrome, certain porphyrias, Hartnup syndrome and pseudoporphyria cutanea tarda. The excision repair disorders are discussed on pp. 191–194. Defects in pigmentation due to an absence of melanocytes (vitiligo) and defective functioning of melanocytes (albinism) also confer susceptibility to UVR because of failure to develop photoprotection through tanning responses.

In idiopathic photodermatoses, the primary abnormality is an acquired alteration in reaction to sunlight. The commonest form is polymorphous light eruption, in which individuals who previously tolerated sun exposure develop itchy papules, vesicles or erythematous patches or plaques on exposed areas after moderate exposure to the sun (Bernhard *et al.*, 1987). Other photosensitivity conditions include solar urticaria (Armstrong, 1986),

hydroa vacciniforme (hydroa aestivale) (Halasz *et al.*, 1983) and actinic reticuloid (Bernhard *et al.*, 1987).

Photoaggravated dermatoses are conditions that may occur in the absence of exposure to sunlight but can be induced or exacerbated by such exposure. The commonest is recurrences of herpes simplex viral eruptions, usually on the upper lip; this viral infection has been reproduced by exposure to artificial sources of UVR (Spruance, 1985).

Other skin diseases reported to be photoaggravated include lupus erythematosus, Darier's disease, acne vulgaris, atopic dermatitis, bullous pemphigoid, disseminated superficial actinic porokeratosis, erythema multiforme, lichen planus, pellagra, pemphigus, pityriasis alba, pityriasis rubra pilaris, psoriasis, acne rosacea, seborrheic dermatitis and transient acantholytic dermatitis (Grover's disease) (Bernhard *et al.*, 1987).

(*b*) *Experimental systems*

Agin *et al.* (1981b) found that single exposures to UVA plus UVB caused thickening of the whole epidermis and stratum corneum in pigmented and albino hairless mice. Sterenborg *et al.* (1986) found similar changes after repeated exposures to mainly UVB in hairless albino mice.

C57Bl mice irradiated with UVB daily for 10 days had a four-fold increase in the number of epidermal melanocytes, with increased pigmentation and local thickening of the epidermis (Rosdahl, 1979). A gradual, delayed, three-fold increase in the number of melanocytes also occurred in shielded contralateral ears, without increased pigmentation or epidermal thickening.

Generally consistent observations have been reported on chronic changes (photoageing) in hairless mice (Bissett *et al.*, 1987, 1989; Kligman, 1989). Bissett *et al.* (1987) described the progression of chronic UV damage to the skin in albino hairless Skh:Hr-1 mice irradiated with UVB or UVB plus UVA three times a week for 16 weeks, with a 17-week recovery period. UVB and a combination of UVA and UVB produced similar changes. An early increase in transepidermal water loss was seen, with a doubling of skin thickness and changes in the microtopography of the skin surface with visible skin wrinkling. Dose-dependent histological changes were seen, with thickening and hyperplasia of the epidermis. Dermal elastic fibres thickened and proliferated throughout the upper dermis, and there was a proliferation of fibroblasts, sebaceous cysts and dermal cysts in the upper dermis. By week 16, the skin was clearly elastotic, with thick, tangled masses of elastic fibres in the dermis. Use of a broad-spectrum sunscreen product with a claimed SPF (skin protector factor) of 15 retarded but did not completely prevent the effects of UVB and of UVB plus UVA radiation. Animals exposed to UVB and then allowed to recover for 12 weeks exhibited a zone of clearance of all abnormal elastin from the dermal–epidermal junction to mid-way down the dermis.

Animals exposed to UVA alone for 33 weeks with a recovery period of 18 weeks (Bissett *et al.*, 1987) exhibited a different pattern of changes. Epidermal thickening occurred at a slower rate, there was no increase in water loss; and sagging rather than wrinkling of the skin occurred. There was a very gradual increase in cellularity; focal areas of collagen damage and absence of elastic fibres were seen; the size and number of dermal cysts increased; and there was only slight evidence of recovery after 18 weeks. UVA appeared to accelerate several

changes similar to those that occur with chronological ageing in mice. Using a dual grating monochromator, Bissett *et al.* (1989) examined the action spectra for these changes. Most were similar and occurred in the UVB waveband: wrinkling, glycosaminoglycan increase, collagen damage, elastosis, epidermal thickening, dermal cellularity and dermal inflammatory cell increase. In contrast, the spectrum for skin sagging was very broad, with a maximum near 340 nm. These results suggest that more than one chromophore is involved in UV-induced chronic skin changes.

High doses of UVA (cumulative dose, 3000 J/cm^2) were reported to produce severe elastic fibre hyperplasia, but no large aggregates of elastosis or destruction of collagen, in female Skh-hr 1 albino mice (Kligman *et al.*, 1985; Kligman, 1989). A dose of 13 000 J/cm^2 from a filtered (50% cutoff at about $\leq$ 345 nm) UVA source, however, produced only insignificant changes. Dose–response studies with another UVA source, filtered to remove all radiation below 340 nm, produced some elastin thickening at a total dose of 8000 J/cm^2 as well as increased epidermal proliferation and increased and enlarged dermal cysts (Kligman *et al.*, 1987).

Kligman and Sayre (1991) found that the action spectrum for elastosis in albino hairless mice was similar to that for erythema, except that longer UVA wavelengths (> 330 nm) were less effective for elastosis.

The chronic effect of repeated UV irradiation was also investigated in naked albino Ng/- mice using high total doses (> 20 000 J/cm^2) from a predominantly UVA source (but containing some UVB) administered for 16 h daily for 8.5 months (Berger *et al.*, 1980a). Dermal changes similar to those seen in human actinic elastosis were observed. There was endothelial swelling of dilated small capillary vessels and slight perivascular infiltration. Particularly in the upper dermis, collagen was replaced with an amorphous material that stained faintly with haematoxylin–eosin. Mast cells and a relatively increased number of spindle-shaped fibroblasts were found in the middle and lower dermis. Large aggregates of numerous tangled, thickened fibres with the staining properties of elastic tissue were seen. Electron microscopy showed that elastic fibres were increased in number and size and there was splitting of collagen fibres. Most small blood vessels were dilated, with multiple basal lamina. The elastic tissue changes showed no signs of regression 2.5 months after irradiation had been discontinued, although the epithelial changes regressed over this period.

Similar changes in elastic tissue (Berger *et al.*, 1980b) were found after exposure to a filtered UVA source which contained no UVB, but no alteration of collagen was observed and inflammatory changes were absent. Electron microscopy showed changes similar to those observed in actinic elastosis.

In female, lightly pigmented, hairless Oslo/Bom mice, UVB alone produced moderate elastosis, UVB and UVA together produced a slightly reduced degree of elastosis, but UVB followed by large doses of UVA produced severe elastosis; UVA alone was reported to have no effect (Poulsen *et al.*, 1984). In Skh:Hr 1 albino hairless mice, a combination of UVA and UVB had additive effects (Kligman *et al.*, 1985).

(*c*) *Comparison of humans and animals*

No direct comparison has been reported of the optical properties of whole human and mouse epidermis; however, the available data suggest that the absorption/transmission

spectra are of a similar general shape but have marked quantitative differences. For example, a comparison of data on a graph of effects on human epidermis not previously exposed to UVR (Bruls *et al.*, 1984a) with tabulated data on mouse epidermis not previously exposed (Sterenborg & van der Leun, 1988), generated in the same laboratory, showed that transmission in the mouse was two orders of magnitude greater in the UVC region and one order of magnitude greater in the UVB and UVA regions than in humans. In human and mouse epidermis, prior exposure to UVR resulted in marked decreases in UVR transmission. No study has been reported on mouse stratum corneum.

4.2.2 *Immune response*

Exposure to solar radiation and UVR can alter immune function in experimental animals and humans. This area of research is known as photoimmunology and has recently been reviewed (Daynes *et al.*, 1983; Parrish, 1983; Parrish *et al.*, 1983; Bergstresser, 1986; Roberts *et al.*, 1986; Krutmann & Elmets, 1988; Morison, 1989).

(*a*) *Humans*

(i) *Contact hypersensitivity (allergy)*

Exposure of normal subjects to radiation in a tanning solarium which emitted mainly UVA but also UVB radiation reduced allergic reactions to 2,4-dinitrochlorobenzene (Hersey *et al.*, 1983a). Halprin *et al.* (1981) and Nusbaum *et al.* (1983) found that UVB radiation partially suppressed the development of contact allergy to nitrogen mustard in patients with mycosis fungoides and psoriasis. Exposure to UVB was begun prior to treatment with mustard, and the field of exposure to the chemical was included in the area exposed to radiation, so that both a local and systemic effect may have been measured. In both studies, the proportion of patients sensitized to mustard gas was reduced by exposure to UVB radiation, and sensitization, when it did occur, was delayed. [The Working Group noted that the presence of diseases known to influence the immune system makes the findings difficult to interpret.]

Response to 2,4-dinitrochlorobenzene was diminished in sun-damaged skin in subjects previously sensitized to the allergen (Kocsard & Ofner, 1964; O'Dell *et al.*, 1980). UVB-induced suppression of contact allergy to nickel and other allergens (e.g., cobalt) has also been reported (Mørk & Austad, 1982; Sjövall & Christensen, 1986).

Studies on the possible mechanism of suppression have focused mainly on the effects on antigen presentation in the skin. At low doses of UVB ($\leq$ 15 mJ/cm^2), Langerhans' cells are the only epidermal cells to be altered morphologically (Aberer *et al.*, 1981). Depletion of Langerhans' cells after a few exposures to UVB radiation is transient (Tjernlund & Juhlin, 1982; Scheibner *et al.*, 1986a); however, chronic exposure to sunlight appears to result in a sustained reduction, since fewer Langerhans' cells are found in exposed than in unexposed skin of older adults but not of young adults (Gilchrest *et al.*, 1982; Scheibner *et al.*, 1983; Thiers *et al.*, 1984; Czernielewski *et al.*, 1988). Pigmentation does not seem to protect Langerhans' cells, since exposure to UVB plus UVA radiation (simulating natural UVR) produced similar degrees of depletion of these cells in dark-skinned Australian aboriginals and in fair-skinned people of Celtic descent (Hollis & Scheibner, 1988); Langerhans' cells

were equally affected in fair-skinned and dark-skinned people after multiple exposures to sunlight (Scheibner *et al.*, 1986b).

The antigen-presenting function of Langerhans' cells is also diminished after irradiation *in vivo* with UVB (Cooper *et al.*, 1985; Räsänen *et al.*, 1989). The function returns to the epidermis within 24 h, owing to the appearance of two cell populations that are distinct and different from Langerhans' cells (Cooper *et al.*, 1986). Both populations have receptors for the monoclonal OKM5 antibody; one also has receptors for the OKM1 antibody and is possibly a dendritic cell from blood, while the other is $OKM1^-$ and is related to a subset of blood monocytes. These cells can activate T cells in the absence of exogenous antigen and lead to the generation of T-suppressor cells which can inhibit various immune responses. Baadsgaard *et al.* (1988) showed that epidermal cells from UVB-irradiated skin can stimulate suppressor/cytotoxic lymphocytes. This may occur *via* at least two pathways: activation of T-suppressor/inducer cells or induction of interleukin-2 production. These observations suggest that UV-induced immune suppression is more closely related to the appearance of $OKM5^+$ cells in the epidermis than to the disappearance of Langerhans' cells.

Systemic suppression of contact allergy may also result from exposure to UVR. Granstein and Sauder (1987) exposed subjects to a MED of mainly UVB radiation and measured levels of serum interleukin-1 activity that peaked 1–4 h after exposure and returned to baseline by 8 h. This activity may originate from the skin, in which increased levels have been detected after UVB irradiation (Kupper *et al.*, 1987; Oxholm *et al.*, 1988; Räsänen *et al.*, 1989).

A recent study (Yoshikawa *et al.*, 1990) showed that suppression of UVB-induced contact allergy may be a risk factor for nonmelanocytic skin cancer. Approximately 60% of normal subjects were sensitized by application of 2,4-dinitrochlorobenzene to UVB-irradiated skin compared to 8% of patients with a history of skin cancer. Many skin cancer patients were also immunologically tolerant to this allergen; this was not observed in normal subjects.

Pigmentation does not protect against UV-induced immunosuppression, since it occurs in the same proportion of black and white people (Vermeer *et al.*, 1991).

(ii) *Lymphocytes*

A single, whole-body exposure to UVB radiation which produced painful erythema produced a transient decrease in the proportion of circulating E rosette-forming cells and in the response of lymphocytes to a mitogen (Morison *et al.*, 1979a). McGrath *et al.* (1986) found a decrease in the proportion of circulating suppressor cells following exposure to half the MED of UVB, although the total number of T lymphocytes was not altered. Exposure of normal subjects to sunlight daily for two weeks, however, produced different effects: The total proportion of T lymphocytes was diminished owing to a pronounced drop in the proportion of helper/inducer cells associated with an increase in the proportion of suppressor cells in the peripheral blood (Hersey *et al.*, 1983b). Similar changes occurred after exposure of normal subjects to UVA plus UVB radiation (Hersey *et al.*, 1983a). When UVB radiation was removed by a Mylar filter (Hersey *et al.*, 1988) or a sunscreen (Hersey *et al.*, 1987), most of the effect was removed. The numbers of circulating T cells and helper-T cells were significantly reduced by exposure of normal subjects to solar lamps containing UVA (with minimal UVB)

and to fluorescent tubes emitting mainly visible light, which contained small quantities of UVB, but the number of T-suppressor cells was only slightly reduced. These effects were considered to be due to the UVB radiation (Rivers *et al.*, 1989).

(iii) *Infectious diseases*

Recurrent infections due to herpes simplex virus types 1 and 2 can be induced by exposure to UVB radiation (Wheeler, 1975; Spruance, 1985; Klein & Linnemann, 1986; Perna *et al.*, 1987). Presumably, local alterations of immunity, associated with extensive UV-induced tissue damage, are responsible for this reactivation.

(iv) *Photosensitive disease*

An interaction between solar radiation and the immune system was first postulated on the basis of observations that the pathogenesis of several diseases is characterized by photosensitivity. Solar urticaria, photoallergy and lupus erythematosus are the main examples (for reviews, see Morison, 1983b,c; Morison & Kochevar, 1983).

(*b*) *Experimental systems*

(i) *Contact hypersensitivity*

The first report of UV-induced suppression of contact hypersensitivity was in guinea-pigs that received applications of a sensitizing chemical through UV-irradiated skin (Haniszko & Suskind, 1963). This effect has since been termed local suppression of contact hypersensitivity. Later, in studies of UV-induced tumour susceptibility in mice, it was found that UVR could also induce systemic suppression of contact hypersensitivity when the sensitizer is applied through unexposed skin only (Kripke *et al.*, 1977). This occurred during chronic treatment of mice, was transient and appeared to be due to failure of an effector mechanism (efferent block) of the immune response (Jessup *et al.*, 1978). These two phenomena, local and systemic suppression of contact hypersensitivity, are probably mediated by different mechanisms.

Local suppression of contact hypersensitivity: Pretreatment of mice with low doses of UVB radiation (100–700 J/m^2 fluorescent sunlamp radiation daily for four days) suppressed the development of contact hypersensitivity to sensitizing chemicals (e.g., 2,4-dinitrofluorobenzene) applied subsequently to irradiated skin (Toews *et al.*, 1980; Elmets *et al.*, 1983). This effect was associated with generation of hapten-specific LyT-1$^+$ T cells which suppress the induction phase of the immune response (Elmets *et al.*, 1983). The most effective wavelengths are < 300 nm (Elmets *et al.*, 1985). Local suppression of contact hypersensitivity by UVB radiation also occurs in hamsters (Streilein & Bergstresser, 1981).

Several hypotheses have been explored to explain the mechanism of local suppression. Multiple exposures to sunlight result in a striking reduction in the number of Langerhans' cells in guinea-pigs, as detected by ultrastructural examination (Fan *et al.*, 1959). UV-induced alterations occur in Ia$^+$ Langerhans' cells (Streilein *et al.*, 1980; Perry & Greene, 1982; Gurish *et al.*, 1983; Stingl *et al.*, 1983), but alterations in other cells may be involved.

Thy-1$^+$ dendritic epidermal cells (identified by antibodies to surface markers on lymphocytes), found in mouse but not reported in human skin, are bone marrow-derived lymphocytes which down-regulate contact hypersensitivity. They are not affected by low-

dose UVR, and hapten-conjugated Thy-1$^+$ dendritic epidermal cells can induce tolerance on subcutaneous injection into the footpad or after intravenous injection (Welsh & Kripke, 1990). This finding is supported by the observations (Okamoto & Kripke, 1987) that (i) the draining lymph nodes of mice treated with low doses of UVR contained these hapten-conjugated cells after exposure to a contact sensitizer, (ii) injection of these cells into other syngeneic mice resulted in the generation of suppressor cells, and (iii) removal of these cells from the lymph node cells abolished the suppression.

I-J$^+$, Thy-1$^-$, Ia$^-$ antigen-presenting cells, which are also resistant to low doses of UVB radiation and preferentially generate a suppressor cell pathway, may also be involved in local suppression (Granstein *et al.*, 1984; Granstein, 1985; Granstein *et al.*, 1987; Okamoto & Kripke, 1987).

Keratinocytes may also be involved through the production of epidermal cell-derived thymocyte-activating factor (ETAF), which is functionally and biochemically very similar to interleukin-1, a nonspecific helper factor necessary for activation of T cells by antigen. Interleukin-1 can reduce expression of contact hypersensitivity in mice (Robertson *et al.*, 1987). Studies by several workers have suggested that exposure to UVR inhibits the production of ETAF (Sauder *et al.*, 1983) or decreases its activity (Stingl *et al.*, 1983). When antigen-presenting cells are exposed to UVR, their ability to activate T cells is markedly inhibited (Tominaga *et al.*, 1983). UV irradiation of mice induces the release of a specific interleukin-1 inhibitor, keratinocyte-derived, EC-contra IL 1 (Schwarz *et al.*, 1988). Other workers (Ansel *et al.*, 1983; Gahring *et al.*, 1984) have found increased production of ETAF. [The Working Group noted that differences in the radiation sources and model systems could explain the discrepancies between the results of these studies.]

Systemic suppression of contact hypersensitivity: Systemic suppression of contact hypersensitivity in mice requires a higher exposure dose (40–50 KJ/m^2) than local suppression (Kripke & Morison, 1986a). A dose of 8.2 kJ/m^2 at 320 nm produced nearly 50% systemic suppression, and 100 kJ/m^2 produced 80% suppression (Noonan *et al.*, 1984). Like local suppression, systemic suppression is associated with the generation of suppressor Lyt-1$^+$ T lymphocytes (Noonan *et al.*, 1981a; Ullrich & Kripke, 1984). The pathways leading to the appearance of these lymphocytes are, however, probably different. Systemic suppression has also been induced in guinea-pigs (Morison & Kripke, 1984) and in the South American opossum, *Monodelphis domestica* (Applegate *et al.*, 1989). Artificial sources of UVB radiation and sunlight, but not UVA, induce systemic suppression of contact allergy in mice and guinea-pigs (Morison *et al.*, 1985).

Determination of an action spectrum for systemic suppression of contact hypersensitivity in mice revealed peak activity in the 260–270 nm region, which is consistent with a superficial location of the chromophore in the epidermis (De Fabo & Noonan, 1983; Noonan & De Fabo, 1985). Two candidate molecules, urocanic acid and DNA, have been suggested.

Several lines of evidence indicate that abnormalities in Langerhans' cells are not involved in systemic suppression, in contrast to local suppression (Lynch *et al.*, 1983; Morison *et al.*, 1984; Noonan *et al.*, 1984), and that a defect of antigen presentation is not an initial step (Kripke & McClendon, 1986). Soluble mediators are released from irradiated skin and may generate suppressor cells in a distant organ. Serum collected from UV-exposed mice and epidermal cells exposed to UVR *in vitro* contain factors that can induce systemic suppression

(Schwarz *et al.*, 1986). The situation is far from straightforward, however, since a recent study indicated that multiple suppressive factors, with different immunosuppressive properties, may be released by different wavelengths of UVR (Kim *et al.*, 1990). Indomethacin blocks the development of suppression (Chung *et al.*, 1986; Jun *et al.*, 1988), indicating that prostaglandins may also be involved in the pathway.

Several properties of the suppressor cells have been defined: (i) they suppress primary proliferative responses but not a secondary response *in vitro* (this is consistent with the idea that they suppress induction of sensitization but not with the proposal that they elicit a response in a previously sensitized animal) (Ullrich, 1985); (ii) their action is limited to T-dependent antigens (Ullrich, 1987); and (iii) they can modulate other immunological pathways, such as formation of anti-hapten antibodies and cytotoxic-T lymphocytes (Ullrich *et al.*, 1986a).

(ii) *Delayed hypersensitivity to injected antigens*

Systemic suppression of delayed hypersensitivity was induced by UVB irradiation of mice following injection of 2,4-dinitrochlorobenzene into the footpad (Jessup *et al.*, 1978), of hapten-coupled spleen cells into the footpad (Greene *et al.*, 1979) or the ear (Noonan *et al.*, 1981b) or of erythrocytes and soluble protein antigens into the footpad (Ullrich *et al.*, 1986b) and is associated with the generation of antigen-specific T lymphocytes. This suppression differs from the suppression of contact hypersensitivity to topically applied allergens because delayed hypersensitivity can be restored in UV-irradiated mice by injection of hapten-coupled spleen cells from normal mice (Noonan *et al.*, 1981b; Kripke & Morison, 1985, 1986b). Furthermore, systemic injection of methylprednisolone before immunization prevented suppression of delayed hypersensitivity but had no effect on the suppression of contact hypersensitivity (Kripke & Morison, 1986b).

Systemic depression of splenic antigen-presenting cell function was demonstrated in UVB-exposed mice (Letvin *et al.*, 1980a,b; Gurish *et al.*, 1982). Two explanations have been advanced: a transient redistribution of antigen-presenting cells to peripheral lymphoid tissues in response to UV-induced inflammation (Gurish *et al.*, 1982; Spangrude *et al.*, 1983) or direct damage to blood monocytes or other precursors of splenic antigen-presenting cells as they circulate through the skin (Spangrude *et al.*, 1983). The latter theory is supported by the observation that immunization with hapten-conjugated splenic antigen-presenting cells or epidermal cells exposed *in vitro* to UVR can induce hapten-specific T-suppressor cells (Fox *et al.*, 1981; Sauder *et al.*, 1981).

The role of one of the proposed chromophores, urocanic acid, has been explored. UV-irradiated urocanic acid (containing 74% *cis*-urocanic acid after 4 h) suppresses delayed hypersensitivity to HSV-1 when injected subcutaneously or applied to the skin of mice (Ross *et al.*, 1986), and is thus similar to UVB radiation (Ross *et al.*, 1987). In both instances, phenotypically similar suppressor cells were induced (Howie *et al.*, 1986a; Ross *et al.*, 1987). In addition, intravenous administration of *cis*-urocanic acid impairs antigen-presenting cell function in splenic dendritic cells. These observations suggest that *trans*-urocanic acid is the photoreceptor for UVB-induced systemic suppression of delayed hypersensitivity and that *cis*-urocanic acid acts as an immunomodulator (Noonan *et al.*, 1988).

(iii) *Immunology of ultraviolet-induced skin cancer*

Most UV-induced tumours in mice are highly antigenic and are rejected upon transplantation into normal syngeneic recipients; however, they grow progressively in immunosuppressed recipients (Kripke, 1974). The specific immunological rejection of these transplanted tumours is mediated by cytolytic-T lymphocytes aided by natural killer and cytotoxic-T cells (Fortner & Kripke, 1977; Fortner & Lill, 1985; Streeter & Fortner, 1988a,b). Tumours grow in UV-irradiated recipients or primary hosts because T-suppressor lymphocytes induced by the exposure to UVR block the normal immunological surveillance system (Fisher & Kripke, 1977; Spellman *et al.*, 1977; Fisher & Kripke, 1978; Spellman & Daynes, 1978). The function of these suppressor cells is specific in that, whereas they prevent development of UVR-induced tumours, they do not alter the growth of chemically induced tumours or skin allografts (Kripke & Fisher, 1976; Fisher & Kripke, 1978).

The phenotype of the suppressor cells is $LyT1^+ 2^-$, Ia^- (antibodies to surface markers on lymphocytes), similar to that of other UV-induced suppressor cells (Ullrich & Kripke, 1984). These suppressor cells are important in the development of primary neoplasms. de Gruijl and van der Leun (1982b, 1983) found accelerated development of UVR-induced tumours in hairless mice that had been exposed previously to UVR at a separate site. Fisher and Kripke (1982) observed that, if suppressor cells were present from the time of commencement of exposure to UVR, the latent period for development of tumours was shortened and the tumour yield was increased. Thus, photocarcinogenesis in mice appears to involve at least two UVR-induced alterations: (i) an alteration in DNA leading to transformation of cells (see pp. 188–189) and (ii) a specific systemic immunological alteration that permits expression of the tumour (Fisher & Kripke, 1977).

Suppressor cells can be induced by doses of 40–50 kJ/m^2 of radiation from fluorescent sunlamps (see Fig. 9c, p. 64) (Kripke & Morison, 1986a), and susceptibility to transplanted tumours is evident long before the de-novo appearance of tumours (Fisher & Kripke, 1977). Suppressor cells can be induced by exposure to UVC (from low-pressure mercury discharge lamps) (Lill, 1983), UVB (De Fabo & Kripke, 1980), large doses of UVA (Morison, 1986) and sunlight (Morison & Kelley, 1985). Wiskemann *et al.* (1986) described an effect of neutral white fluorescent bulbs. [The Working Group considered that this effect may have been due to low levels of UVB from this source.]

(iv) *Transplantation immunity*

The immune responses in graft rejection and graft-*versus*-host disease are complex and directed against class I antigens of the major histocompatibility complex which are expressed on all nucleated cells and class II Ia antigens which are expressed normally on lymphocytes and macrophages. Lindahl-Kiessling and Säfwenberg (1971) demonstrated that UV irradiation of stimulator cells could abrogate the proliferation of responder cells in a mixed-lymphocyte reaction. Subsequent studies (Alter *et al.*, 1973; Bach *et al.*, 1977) indicated that this effect was due to alteration of class II Ia antigens on the cells bearing them. These initial observations have been extended to various systems.

Pre-transplant, donor-specific blood transfusions have been used to reduce the need for post-transplant immunosuppression, with varying success. The basis for this effect is thought to be generation of donor-specific T-suppressor lymphocytes in the host. Lau *et al.* (1983)

found that exposure of the blood to UVB radiation prior to transfusion greatly enhanced this effect and permitted long-term survival of allografts of islets of Langerhans across a major histocompatibility barrier in rats. The effect was shown to be due to inactivation of lymphocytes by radiation, resulting in cancellation of a signal from Ia antigen-positive cells and permitting the generation of donor-specific T-suppressor cells. A similar effect was demonstrated with rat heart allografts (Balshi *et al.*, 1985).

Deletion of Ia antigens or inactivation of cells bearing them may explain prolonged graft survival in other systems. Exposure of mouse tail skin to UVB radiation *in vitro* prolonged its survival as a graft when I-region differences only were present, but UVB had no effect in the case of complete H-2 differences (Claas *et al.*, 1985). Similarly, mouse corneal allograft survival was prolonged by exposure to UVB radiation *in vitro* (Ray-Keil & Chandler, 1986). Prolonged survival as grafts of rat islets of Langerhans exposed to UVB radiation *in vitro* was apparently due to inactivation of dendritic cells bearing Ia antigens (Lau *et al.*, 1984).

The model of UVR-induced systemic suppression of delayed hypersensitivity has been extended to transplantation studies, because of the considerable potential for manipulating the immune system in transplantation. Sensitization of mice with allogeneic spleen cells after a single exposure to UVB radiation suppressed the delayed hypersensitivity response to these cells and proliferation of lymphocytes from the irradiated mice in a mixed-lymphocyte reaction; these effects are due to generation of suppressor cells specific for donor antigens (Ullrich, 1986). Interestingly, exposure of the mice to radiation need not precede exposure to the antigen but can be delayed up to five days after first contact with the antigen, unlike other forms of suppression of delayed hypersensitivity (Magee *et al.*, 1989a). Similar observations have been made in rats, but suppressor cells were not demonstrated in the spleen (Magee *et al.*, 1989b). Subcutaneous injection of epidermal cells that have been exposed to UVB radiation *in vitro* can similarly cancel a delayed hypersensitivity response in mice; this effect is associated with prolongation of skin allograft survival (Tamaki & Iijima, 1989).

Graft-*versus*-host disease can also be reversed by UVR. Two rat models have been studied. Pretreatment of donor bone marrow with UVB radiation did not increase the failure of grafts, but it prevented graft-*versus*-host disease in most instances (Pepino *et al.*, 1989). Pre-irradiation of rat skin with UVB prevented subsequent development of cutaneous graft-*versus*-host disease at the site of exposure (Glazier *et al.*, 1984). In both of these studies, an alteration of Ia-bearing cells was postulated as the mechanism.

(v) *Infectious diseases*

Classic delayed hypersensitivity to complex protein antigens (correlated with resistance to a number of infections) can be suppressed by exposure to UVB radiation (Ullrich *et al.*, 1986b).

Exposure of mice to low doses (1.3–3.4 kJ/m^2) of UVB (less than a human MED) at the site of intradermal infection with herpes simplex type 2 virus increased the severity of the disease. Unirradiated mice developed only a single vesicle at the site of inoculation, whereas irradiated mice developed zosteriform lesions which healed slowly and, at the highest dose of radiation, were lethal. At doses that increased the severity of the infections, systemic suppression of delayed hypersensitivity to the virus due to generation of antigen-specific T-suppressor lymphocytes was observed (Yasumoto *et al.*, 1987). In-vitro assays showed

UVB-induced impairment of antigen presentation, which may have been due to the presence of suppressor factors in the supernatant (Hayashi & Aurelian, 1986). Similar results were found in a model of herpes simplex virus type 1 infections in mice (Howie *et al.*, 1986a,b,c; Otani & Mori, 1987). [The Working Group considered that these experiments have not demonstrated clearly that the effect of radiation on the induction of immunity is local, since the possibility of an indirect systemic effect has not been explored.]

Exposure to low doses of UVB radiation prevented the development of delayed hypersensitivity to the protozoan, leishmania, and reduced the number and severity of skin lesions when leishmania was inoculated at the site of exposure. Exposure to radiation did not, however, alter the viability of the organisms or the degree of their dissemination to distant sites—the spleen, lymph nodes and skin. Furthermore, the irradiated mice reacted to a second, distant inoculation as if it were a primary infection, presumably because they lacked the cell-mediated immunity that would be needed to control this second attack of the organism (Giannini, 1986).

Exposure of mice to UVB radiation also caused systemic suppression of delayed hypersensitivity to the yeast *Candida albicans* (Denkins *et al.*, 1989), through two possible mechanisms: one mediated by suppressor cells (detected in the spleen) triggered by exposure to radiation prior to contact with the antigen and another which did not involve splenic suppressor cells and was triggered by exposure to radiation following exposure to the antigen.

(vi) *Human lymphocytes* in vitro

Lymphocytes are highly sensitive to low doses of UVR. UVC was approximately 10 times more effective than UVB and 10^5 times more effective than UVA on mononuclear peripheral blood cells *in vitro* (Morison *et al.*, 1979b). Cripps *et al.* (1978) found that UVC was preferentially toxic to T lymphocytes, but that T and B lymphocytes were similarly susceptible to UVB. UVA did not appear to kill T or B cells. Exposure of mononuclear peripheral blood cells to UVB radiation inhibited both natural killer cell activity and the response of these cells to stimulation by a mitogen (phytohaemagglutinin) (Schacter *et al.*, 1983), in the absence of any apparent change in viability. The effect on natural killer cell activity occurred selectively at the post-binding stage of lysis (Elmets *et al.*, 1987) and could be virtually reversed by the addition of interleukin-2 and superoxide dismutase (Toda *et al.*, 1986).

(*c*) *Comparison of humans and animals*

Firstly, most observations have been made in experimental systems and few studies have involved humans, and it can be only assumed that results of studies in mice can be extrapolated to humans. Furthermore, in no instance have parallel studies in an experimental system and in humans been performed to test this assumption. Secondly, while most investigations of photoimmunology have focused on the effects of 'UVB' radiation, in most studies this term refers to the emission spectrum of a fluorescent sunlamp (see Fig. 9c, p. 64) which contains both UVC and UVA, as well as UVB radiation, besides having little in common with the spectrum of sunlight. Fortunately, in the few studies in which the effects of fluorescent sunlamps and sunlight have been compared in experimental systems, similar alterations in immunity have been observed. Finally, with few exceptions, the effect of

exposure to UVR is to suppress immunity highly selectively, at least in experimental animals. Thus, in mice, certain cell-mediated immune responses are suppressed by UVR, whereas humoral immunity is largely unaffected. The selective nature of UVR-induced immunosuppression has not been established in humans, but no evidence exists to suggest that it does not apply. The importance of such selectivity is that it differs from the forms of immunosuppression seen most commonly in humans, namely viral and drug-induced suppression, which affect most functions of the immune system. Exposure of humans to UVR is unlikely to cause paralysis of immune function but probably selectively negates a few immune responses.

4.2.3 *Eye*

(*a*) *Humans*

(i) *Anterior eye (cornea, conjunctiva)*

The cornea absorbs UVC and UVB radiation (Sliney & Wolbarsht, 1980). Sunlight has been implicated as causing nodular band keratinopathies (spheroidal degeneration and climatic droplet keratopathy), pinguecula, pterygium, photokeratitis and photokeratoconjunctivitis (Wittenberg, 1986). Artificial sources of UVR, including welding arcs and germicidal lamps, cause photokeratoconjunctivitis and photokeratitis (Sliney, 1986). A study by Taylor *et al.* (1989) of the association between exposure to broad-band UVR and corneal disease in 838 fishermen in Chesapeake Bay, Maryland, USA, reported a significant association with pterygium and climatic droplet keratopathy but a weak association with pinguecula.

(ii) *Lens*

The lens absorbs radiation between 305 and 400 nm (Wittenberg, 1986). UVR produces substantial photodamage to both the structural proteins and key enzymes of the lens (for review, see Andley, 1987).

Taylor *et al.* (1988) studied the two major types of senile cataract (nuclear and cortical cataracts) in 838 Maryland fishermen for each of whom mean annual and cumulative UVB exposure had been assessed. High cumulative exposure to UVB and high annual exposure to UVB were both associated with increased risk of cortical cataract, but no association was seen with nuclear cataracts. The association between exposure to solar radiation and cataract is also supported by studies of cataract in northern India and China and in aborigines in Australia and by an analysis of data from the US National Health and Nutritional Examination Survey. These studies were reviewed by Wittenberg (1986).

It has been claimed that the presence of low levels of photosensitizing compounds in lens tissue may contribute to cataractogenesis (Lerman, 1988).

(iii) *Posterior eye*

The posterior eye is composed of the vitreous humour and the retina (Lerman, 1980). In the normal eye, solar radiation in the visible and near infrared regions (400–1400 nm) reaches these structures. Refraction of this waveband by the cornea and lens greatly increases the irradiance between the surface of the cornea and the retina (Sliney & Wolbarsht, 1980).

Permanent retinal damage was observed after direct viewing of the sun and viewing of solar eclipses and in aircraft spotters during the Second World War, but no epidemiological

study has associated retinal pathology with routine environmental exposure to sunlight (Wittenberg, 1986). The suggestion that senile macular degeneration is related to solar exposure was not supported by a large study of fishermen in Maryland (West *et al.*, 1989).

(*b*) *Experimental systems*

(i) *Anterior eye*

Pitts *et al.* (1977) and Cullen (1980) studied the effects of exposure to UVR at 295 nm on the corneas of pigmented rabbit eyes. The threshold dose for corneal damage was 0.05 J/cm^2. Changes observed with a slit lamp biomicroscope included discharge, corneal debris, haziness, granular change, epithelial exfoliation, stromal opacities and stromal haze.

Applegate and Ley (1991) showed that UVR-induced corneal opacification and neovascularization of the cornea of the South American opossum *M. domestica* was due to DNA damage, as these effects could be delayed by subsequent illumination with photoreactivation light, which specifically monomerizes pyrimidine dimers.

(ii) *Lens*

Cataracts have been produced in pigmented rabbit eyes by exposure to UVB radiation (Pitts *et al.*, 1977). Cataracts were produced in young albino mice 60 weeks after irradiation with a black light (predominantly UVA) (Zigman & Vaughan, 1974; Zigman *et al.*, 1974). Albino mice developed anterior lens opacities after daily exposure for one to two months to a UVB plus UVA source (290–400 nm), but not after the source was filtered to remove radiation < 320 nm (Jose & Pitts, 1985).

(iii) *Posterior eye*

The effects of solar radiation on the posterior eye have been reviewed (Wittenberg, 1986; Andley, 1987). Irradiation of calf vitreous humour *in vitro* with visible radiation in the presence of photosensitizers resulted in partial liquefaction, suggesting that photogenerated active species of oxygen may damage the vitreous structure. In rabbits *in vivo*, however, little liquefaction was seen, suggesting a protective mechanism in the intact organ (Pitts *et al.*, 1977).

Damage to the retina by exposure to sunlight may also be due to thermal effects at high irradiances or to photochemical effects at lower irradiances. In various animals, continuous exposure to sunlight produces a photochemical lesion involving the entire retina and affecting both rods and cones (Young, 1988). The photopigment, rhodopsin, is the chromophore for damage to the rods, while the three cone pigments are the chromophores for cones. In monkeys, blue-light damage caused by exposure to the 400–500 nm waveband affected the macular or paramacular region of the retinal pigment epithelium. The chromophore involved has been postulated to be melanin; active species of oxygen appear to act as mediators of the photochemistry (Lerman, 1980; Andley, 1987).

(*c*) *Comparison of humans and animals*

The limited data available indicate that the optical properties of the components of human and animal eye are broadly similar.

4.3 Photoproduct formation

4.3.1 *DNA photoproducts*

A multitude of photoproducts are formed in cellular DNA by solar UVR, many of which were first recognized after their induction by non-solar radiation at a wavelength of 254 nm. The ratio of the different photoproducts changes markedly with wavelength. A brief description of the photoproducts is given below, together with a note on the wavelength dependence of formation and susceptibility to repair. Substantial information on biological consequences is available only for cyclobutane-type pyrimidine dimers and pyrimidine–pyrimidone (6-4) photoproducts.

(*a*) *Cyclobutane-type pyrimidine dimers*

Shortly after the observation that thymine compounds irradiated with UVC in the frozen state rapidly lose their absorption (Beukers *et al.*, 1958), a dimer of thymine was shown to be responsible for this effect, the two molecules being linked by a cyclobutane ring involving the 5 and 6 carbon atoms (Beukers & Berends, 1960; Wulff & Fraenkel, 1961). Continued irradiation leads to a wavelength-dependent equilibrium between dimer formation and dimer splitting to reform the monomer. Dimer formation is favoured when the ratio of dimer to monomer absorbance is relatively small (wavelengths > 260 nm), whereas monomerization is favoured at shorter wavelengths (around 240 nm), when the ratio is larger (Johns *et al.*, 1962). Although several isomers of the cyclobutane-type thymidine dimer have been isolated from irradiated thymine oligomers, only the *cis*-syn isomer appears to predominate in biological systems (Ben-Hur & Ben-Ishai, 1968; Varghese & Patrick, 1969; Banerjee *et al.*, 1988).

Cytosine–thymine (cyt↔thy), thymine↔thymine (thy↔thy) and cytosine–cytosine (cyt↔cyt) cyclobutane-type dimers are also formed in irradiated *Escherichia coli* DNA but deaminate to uracil↔thymine (ura↔thy) and uracil–uracil dimers after the acid hydrolysis usually used in chromatographic analysis (Setlow & Carrier, 1966). Cytosine moieties in dimers are also deaminated at a slower rate under physiological conditions that produce uracil residues (Fix, 1986), and recent evidence obtained in bacteria suggests that the rate may be more significant than was previously thought (Tessman & Kennedy, 1991). After treatment at 254 nm, thy↔thy, cyt↔thy and cyt↔cyt appear in irradiated DNA at a ratio of 2:1:1 (Unrau *et al.*, 1973), but this ratio changes quite markedly at longer wavelengths, e.g., to 5:4:1 at 265 nm (Setlow & Carrier, 1966). At 254 nm, the relative proportion of cyclobutane dimers was: 5′-thy↔thy, 0.68; 5′-cyt↔thy, 0.17; 5′-cyt↔thy, 0.08; and 5′-cyt↔cyt, 0.07 (Kraemer *et al.*, 1988). Ellison and Childs (1981) showed in *E. coli* that the ratio of cyt↔thy:thy↔thy increases from 0.75 at 254 nm to 1.5 at 313 nm then decreases to 0.8 at 320 nm, the longest wavelength tested. At 365 nm, the longest wavelength at which dimers have been detected, the ratio of thy↔thy:ura↔thy was 5–6:1 (Tyrrell, 1973). The proportion of cyt↔cyt:thy↔thy increased up to 300 nm, but cyt↔cyt was undetectable at longer wavelengths (Ellison & Childs, 1981). On the basis of these data, the latter authors argued that the predominant dimer species formed in *E. coli* by exposure to sunlight are likely to be mixed dimers of cyt↔thy rather than thy↔thy (cyt↔thy:thy↔thy, 1.2:1). The ratio of formation of thy↔thy:ura↔thy dimers in bacterial DNA at 254 and 365 nm is approximately 7×10^5 nm

(Tyrrell, 1973). A similar ratio of total dimer product formation was found in cultured human skin fibroblasts irradiated at 254–265 nm (Enninga *et al.*, 1986).

Fisher and Johns (1976) described the photochemistry and mechanism of formation of cyclobutane-type pyrimidine dimers in considerable detail. The mechanism of dimer formation in the UVB region almost certainly involves direct absorption, since the action spectrum for induction closely resembles that for the appropriate monomer for wavelengths as long as 313 nm (Ellison & Childs, 1981). The mechanism of formation by longer wavelengths (e.g., 365 nm) has not been clarified.

Cyclobutane-type dimers can be removed from the DNA of both prokaryotic and eukaryotic cells by the powerful excision repair mechanism that is deficient in cells from most sun-sensitive, skin cancer-prone patients with the hereditary disease, xeroderma pigmentosum (see Friedburg, 1984; Cleaver & Kraemer, 1989). Photoreactivation is specific for pyr↔pyr (pyrimidine dimers) and monomerizes them *in situ via* a photolyase. Many microorganisms and higher eukaryotes contain a photolyase, but the proteins and light-activation spectra differ from species to species. The specificity of this process has proved a powerful tool in analysing the role of pyr↔pyr in biological effects. For example, the potential photoreactivation of pyr↔pyr has been studied in a set of experiments to demonstrate that the presence of UVC-induced pyr↔pyr in fish can be a precarcinogenic lesion (Setlow, 1975). More recently, the small opossum, *M. domestica*, has been used by Ley and coworkers as an animal model in studies on the effects of UVR, predominantly UVB, mainly because cells of the skin of this animal, unlike that of the mouse, contain a photoreactivating enzyme(s). They showed that several biological effects, including decreased hair growth, erythema and tumour formation, were suppressed by exposure to longer wavelengths (photoreactivating light) (Ley & Applegate, 1989; Ley *et al.*, 1991).

Considerable evidence, including the fact that photoreactivation prevents formation of the majority of mutations induced in bacteria by UVC, shows that the argument that pyr↔pyr is a major premutagenic lesion is overwhelming (Doudney, 1976). Recognition that UV-induced mutagenesis in bacteria is an inducible process (see Witkin, 1976), however, complicates this argument, since, assuming that a structure involving pyr↔pyr constitutes the inducing event, its elimination by photoreactivation would preclude error-prone repair at the site of any premutagenic lesion. When all inducible functions relevant to mutagenesis are turned on, the photoreversibility of UVC mutagenesis at several pyr↔pyr sites disappears (Bridges & Brown, 1992); e.g., UV-induced mutagenesis to *his*$^+$ in certain *rec*A441 *lex*A51 bacteria was not photoreversible, indicating that pyrimidine dimers are not target lesions (Ruiz-Rubio *et al.*, 1986). This suggests that non-photoreversible photoproducts (such as the pyrimidine–pyrimidone 6-4 photoproduct) are the principal premutagenic lesions at dithymine sequences and that cyclobutane-type thymine dimers are weakly mutagenic. This conclusion is consistent with the results of other studies with single-stranded vector DNA containing cyclobutane-type (6-4) thy↔thy photoproducts at specific sites (Banerjee *et al.*, 1988, 1990; LeClerc *et al.*, 1991).

(*b*) *Pyrimidine–pyrimidone (6-4) photoproducts*

The most extensively studied non-dimer photoproduct is that formed from thymine and cytosine. Indirect evidence (Varghese & Patrick, 1969) suggests that this structure is the

in-vivo precursor of the compound 6-4′-[pyrimidin-2′-one]thymine (thy(6-4)pyo), originally found in acid hydrolysates of UV-irradiated DNA (Varghese & Wang, 1967; Wang & Varghese, 1967). Some years later, a type of UV-induced photoproduct, the pyrimidine nucleoside–cytidine lesion, was recognized in highly reiterated sequences of human DNA (Lippke *et al.*, 1981); this is also probably a precursor of the thy(6-4)pyo product (Brash & Haseltine, 1982; Franklin *et al.*, 1982). Using DNA sequencing analysis, UV photoproducts were more frequent at the 3′ end of pyrimidine runs. Although the overall ratio of 6-4 photoproducts to dimers was 15% at certain 5′-thy↔cyt sequences, 6-4 photoproducts occurred at approximately the same frequency as that of the cyclobutane dimer (Kraemer *et al.*, 1988).

Patrick (1977) originally reported that the action spectrum for (6-4) photoproduct formation resembles that for cyclobutane dimer formation, although the quantum yields are two and ten times lower than that of cyt↔thy and thy↔thy formation, respectively. Using irradiation at wavelengths as long as 334 nm, Chan *et al.* (1986) found that the action spectrum for induction of hot alkali sites (presumably the thy(6-4)pyo hydrolysis product) was also similar to that for pyr↔pyr formation. The action spectra for the induction of thymine dimers and (6-4) photoproducts were similar from 180 to 300 nm, whereas the action spectrum values for thymine dimer induction were about nine and 1.4 times higher or more than the values for (6-4) photoproduct induction below 160 nm and above 313 nm, respectively (Matsunaga *et al.*, 1991).

Most xeroderma pigmentosum patients are defective in the excision of (6-4) photoproducts (Mitchell *et al.*, 1985) and cyclobutane pyrimidine dimers (Cleaver & Kraemer, 1989). In addition, a group of patients with trichothiodystrophy (type 3) showed a marked reduction in the repair of (6-4) photoproducts (Broughton *et al.*, 1990).

Glickman *et al.* (1986) demonstrated in *E. coli* that the cytosine–cytosine pyrimidine–pyrimidone (6-4) photoproduct is highly mutagenic; however, in other studies (e.g., Hutchinson *et al.*, 1988), cyclobutane dimers were shown to be responsible for the majority of observed mutations. Assessment of the relative contributions to mutagenesis of all dipyrimidine photoproducts will require comprehensive studies in different biological systems with specifically designed sequences containing the appropriate photoproducts. Both pyrimidine dimers and pyrimidine–pyrimidone (6-4) photoproducts appear to be important in inducing cytotoxic and mutagenic lesions in human cells, although the relative contributions of each type remain controversial (Mitchell, 1988).

(*c*) *Thymine glycols*

A group of monomeric ring-saturated lesions of the 5,6-dihydroxydihydrothymine type (thymine glycols) have been detected by alkaline–acid degradation in the DNA of UV-irradiated human cells (Hariharan & Cerutti, 1976, 1977). Alkaline–acid degradation (see Cerutti, 1981) can be used to detect a class of structurally related lesions rather than a single lesion, with a yield that has been estimated to be approximately 20% of the total of ring-saturated thymine products (t_{sat}).

Two aspects of this class of UV photoproduct are of particular interest: firstly, they are closely related to a class of ionizing radiation products and are believed to arise through a similar mechanism, i.e., indirectly *via* the action of hydroxyl radicals; secondly, their yield (relative to that of other UV-induced base damage) increases with exposures in the UVB

region. Measurements in HeLa cells showed that at 265 nm the ratio of thy↔thy to t_{sat} was 21, whereas at 313 nm the ratio decreased to 1.3 (Cerutti & Netrawali, 1979). The saturated thymine damage induced by UVA and UVB radiation may thus be due to the effects of active oxygen species generated *via* endogenous cell components. There is little evidence pertaining to the lethal or other biological consequences of such lesions in mammalian cells, although a glycosylase capable of repairing these lesions has been isolated from human cells (Higgins *et al.*, 1987).

(*d*) *Cytosine damage*

The photochemical induction of pyrimidine hydrates has been reviewed (Fisher & Johns, 1976). Significant levels of hydrates are probably formed initially by UVR; however, their instability hampers measurement of their induction and removal in cells, and it has not been possible to establish a cause-and-effect relationship between photohydrate induction and biological effects *in vivo*. Using sequencing techniques, Gallagher *et al.* (1989) observed incision by human endonucleases of unidentified cytosine photoproducts that were neither cyclobutane-type nor (6-4) pyrimidine dimers. The frequency of these two photoproducts was two orders of magnitude lower than that of pyrimidine dimers, and the optimal wavelengths for induction were between 270 and 295 nm.

(*e*) *Purine damage*

Purine damage has been studied less frequently than pyrimidine damage, since the quantum yields are at least one order of magnitude lower; however, the development of sequencing techniques has made their detection easier (Kumar *et al.*, 1991). Incisions (endonuclease V) are detected at unidentified purine or purine–pyrimidine moieties after broad-spectrum UV irradiation (Gallagher & Duker, 1986). Such damage appears to be induced maximally in the wavelength region of 260–300 nm (Gallagher & Duker, 1989). Although the overall yield is much lower than that of pyr↔pyr, similar yields occur at certain loci.

(*f*) *DNA strand breaks*

UVC radiation induces a lower proportion of single-strand breaks than of other photoproducts. In contrast, strand breaks are the commonest initial lesion induced by ionizing radiation. Although strand breaks form only a minority of lesions after irradiation at wavelengths up to 365 nm, they become increasingly important at longer wavelengths in the solar UV region (290–400 nm). At 313 nm, the ratio of DNA strand breakage to pyr↔pyr induction in intact *E. coli* was 1:44 (Miguel & Tyrrell, 1983), whereas at 365 nm one strand break was formed for approximately every two pyrimidine dimers (Tyrrell *et al.*, 1974). An action spectrum for break induction in *Bacillus subtilis* DNA *in vivo* is available (Peak & Peak, 1982). More recently, an action spectrum for single-strand breaks in human skin cells has been determined which shows that irradiation in the presence of deuterium (which enhances singlet oxygen lifetime) increases the number of strand breaks observed at 365 and 405 nm. At wavelengths of 405 nm and longer, strand breaks and DNA–protein cross-links are the only forms of photochemical damage that have been determined (Peak *et al.*, 1987). Between 10 and 20% of the breaks induced at 365 nm are not frank breaks but rather alkali-labile

bonds which presumably include apurinic and apyrimidinic sites (Ley *et al.*, 1978; Peak & Peak, 1982). The formation of breaks is strongly dependent upon oxygen at both 313 (Miguel & Tyrrell, 1983) and 365 nm (Tyrrell *et al.*, 1974; Peak & Peak, 1982). Their formation *in vitro* at 365 nm is also quenched by free-radical scavengers. Strand breaks are repaired rapidly by a variety of cellular mechanisms in both prokaryotes and eukaryotes. The role of these lesions in the biological action of solar radiation is not well understood (Tyrrell *et al.*, 1974).

(*g*) *DNA–protein cross-links*

The photochemical addition of nucleic acids to amino acids and proteins both *in vitro* and *in vivo* has been the subject of several reviews (Smith, 1976; Shetlar, 1980). Of the 22 common amino acids, 11 undergo photochemical addition to labelled uracil, the most reactive of which is cysteine, and several heterophotoproducts involving cysteine have been isolated and characterized.

Several prokaryotic and eukaryotic proteins have been cross-linked photochemically to DNA *in vitro*, including DNA polymerase, RNA polymerase, helix destabilizing protein and mixtures of proteins (Shetlar, 1980).

There is evidence that DNA–protein cross-links are formed in mammalian cells in significant yields by wavelengths longer than 345 nm (Bradley *et al.*, 1979; Peak & Peak, 1991). Action spectra for the formation of DNA–protein cross-links in human cells have now been obtained. Two peaks of induction are observed: one at 254–290 nm, corresponding to the peak of DNA absorption, and a second at 405 nm, presumably resulting from a photosensitization reaction (Peak *et al.*, 1985). [The Working Group noted that DNA–protein cross-links are likely to have important consequences for cells, but no data are available to allow evaluation of their effects in eukaryotic cells.]

4.3.2 *Other chromophores and targets*

In addition to DNA, many other cellular components absorb and/or are damaged by solar UVR and may influence the biological outcome of exposure. Both informational and transfer RNA molecules are susceptible to photomodification. Studies in insects indicate that damage to messenger RNA may be relevant to embryonic development, but the relevance of these results to mammalian systems is unclear (Kalthoff & Jäckle, 1982). Detailed results of bacterial studies on the photolability of certain components of transfer RNA (Jagger, 1981) are almost certainly not relevant to mammalian cells. Damage to proteins could lead to modification of the level of persistent primary damage in DNA, such that cellular DNA repair and antioxidant pathways are compromised (Tyrrell, 1991). There is also evidence that components of electron transport and oxidative phosphorylation, as well as membranes and membrane transport systems, can be damaged by solar wavelengths (Jagger, 1985). Non-DNA chromophores and targets become particularly relevant at longer wavelengths.

(*a*) *Chromophores*

Both nucleic acids and proteins weakly absorb UVA, and, although direct photochemical events may occur, it appears likely that the initial event in the biological effects of UVA radiation is absorption by a non-DNA chromophore which results in generation of

active oxygen species or energy transfer to the critical target molecules. As a consequence, at long UV wavelengths, the range of targets is extended to all critical molecules that are susceptible to active intermediates generated by chromophores.

Most of the knowledge on relevant chromophores has been obtained from in-vitro experiments or from studies in bacteria (Eisenstark, 1987). Indirect evidence indicates that porphyrins play a role in the inactivation of *Propionibacterium acnes* by UVA (Kjeldstad & Johnsson, 1986). It has also been shown that *E. coli* mutants defective in the synthesis of δ-aminolaevulinic acid are resistant to inactivation by UVA (Tuveson & Sammartano, 1986), which strongly suggests that porphyrin components of the respiratory chain act as endogenous photosensitizers. This conclusion is supported by the finding that strains that overproduce cytochrome were sensitive to broad-band UVA radiation (Sammartano & Tuveson, 1987). Porphyrins are also essential to human cellular metabolism, and overproduction of iron-free porphyrins in erythropoietic or hepatic tissues is the underlying cause of the photodestruction of the skin seen in the group of diseases known as porphyrias. Although direct evidence is lacking, free porphyrins and proteins containing haem (such as catalase, peroxidases and cytochromes) are also potentially important chromophores in skin cells from normal individuals. Many other cellular compounds which contain unsaturated bonds, such as flavins, steroids and quinones, should also be considered potential chromophores. Although normal levels of catalase (which contains haem) and alkyl hydroperoxide reductase (which contains FAD) would be expected to exert a protective role in bacteria (see below), overproduction of these enzymes is correlated with an increase in sensitivity to UVA radiation in bacteria (Kramer & Ames, 1987).

Porphyrins are an important class of photodynamic sensitizers which are believed to exert their biological action *via* the generation of singlet oxygen. Recent experiments have shown that deuterium oxide (which prolongs the lifetime of singlet oxygen) sensitizes human fibroblast cell populations to the lethal action of UVA radiation, while sodium azide (which destroys singlet oxygen) protects them (Tyrrell & Pidoux, 1989). Although this finding is consistent with the involvement of porphyrins in the lethality of UVA, other cellular compounds may also generate singlet oxygen. It is also important to consider active oxygen species that may be generated intracellularly. Not only can hydrogen peroxide be generated by UVA irradiation of tryptophan (McCormick *et al.*, 1976), but both superoxide anion and hydrogen peroxide can be generated by photo-oxidation of NADH and NADPH (Czochralska *et al.*, 1984; Cunningham *et al.*, 1985).

The presence of chromophores (such as psoralens) in the diet may also influence susceptibility to damage, but this reaction is clearly subject to enormous individual variability. Accidental and deliberate application of chemical agents (such as sunscreens and drugs) to the skin may also introduce potentially damaging chromophores.

(b) Membranes

The lipid membrane is readily susceptible to attack by active oxygen intermediates. Many reports (e.g., Desai *et al.*, 1964; Roshchupkin *et al.*, 1975; Putvinsky *et al.*, 1979; Azizova *et al.*, 1980) have shown that UVR can induce peroxidation of membrane lipids. In-vitro studies with lecithin microvesicles have shown UVR-induced changes in the microviscosity of membrane bilayers (Dearden *et al.*, 1981) which are correlated with the degree of unsatu-

ration of fatty acid chains (Dearden *et al.*, 1985). UVC and UVA radiation and sunlight have been shown to cause lipid peroxidation in the liposomal membrane (Mandal & Chatterjee, 1980). Haem proteins such as cytochrome *c* and catalase are known to catalyse lipid peroxidation and peroxidative breakdown of membranes (e.g., Brown & Wüthrich, 1977; Goñi *et al.*, 1985; Szebeni & Tollin, 1988). A dose-dependent, linear increase in lipid peroxidation of liposomal membranes was induced by UVA radiation, which was inhibited to a large extent by butylated hydroxytoluene, a nonspecific scavenger of lipid-free radicals. Since both sodium azide and L-histidine (quenchers of singlet oxygen) led to 40–50% inhibition of peroxidation, the authors suggested that singlet oxygen is involved in initiation of the reaction (Bose *et al.*, 1989).

UVA irradiation of liposomes leads to lipid peroxidation in the absence of photosensitizer molecules, so that singlet oxygen may arise through direct stimulation of molecular oxygen (Bose *et al.*, 1989). Biological membranes are, however, rich in endogenous photosensitizer molecules, such as those involved in electron transport, and these may contribute to the peroxidation of lipids observed in biological systems (see Jagger, 1985). Membrane damage has long been implicated in the lethality of UVA in bacteria (Hollaender, 1943) and almost certainly contributes to the sensitivity of UVA-treated populations plated on minimal medium—a phenomenon which is highly dependent on oxygen (Moss & Smith, 1981). Sensitivity to UVA has been related to levels of unsaturated fat in membranes (Klamen & Tuveson, 1982; Chamberlain & Moss, 1987). Furthermore, the presence of deuterium oxide enhances the levels of membrane damage, sensitivity to UVA and lipid peroxidation (Chamberlain & Moss, 1987), suggesting that singlet oxygen plays a role in all three processes. Leakage experiments have also been used to assess UVA-induced membrane damage in yeast: again, changes in permeability correlated well with lethality and were highly oxygen dependent (Ito & Ito, 1983). UVA irradiation of cultured human and mouse fibroblasts led to the release of arachidonate metabolites from the membrane in a dose-dependent fashion. The release was also dependent on the presence of both oxygen and calcium ion and may be related to the induction of cutaneous erythema, which is also oxygen dependent (Hanson & DeLeo, 1989). Studies of the effects of UVR on membrane transport have been undertaken in prokaryotes (Jagger, 1985), but no information was available on the effects of UVR on eukaryotic membrane transport.

4.4 Human excision repair disorders

4.4.1 *Xeroderma pigmentosum*

The commonest, most characteristic photoproducts produced in DNA by UVB and UVC radiation involve adjacent pyrimidines. Evidence summarized above argues strongly that these products give rise to a wide variety of alterations in DNA sequence and gene expression. Like many other types of DNA damage, these photoproducts may be excised, and the resulting gap in one strand can be resynthesized accurately using the undamaged strand as a template. How this is accomplished is best understood in the bacterium *E. coli*, in which a multiprotein complex including the products of the *uvr*A, B and C genes excises an oligonucleotide 12 or 13 bases in length containing the photoproduct. The resulting gap is filled by a DNA polymerase (usually III), and the final ligase link to the adjacent DNA is effected by

polynucleotide ligase (Bridges *et al.*, 1987; Bridges, 1988; Bridges & Bates, 1990). Other gene products are involved in the process, and a more comprehensive discussion is given by Sancar and Rupp (1983). Bacteria that have defects in the *uvr*A or B genes cannot excise UV photoproducts and are 10–20 times more sensitive to killing and the induction of mutations by UVC. They are also more sensitive to UVB and (under certain conditions) UVA (Webb, 1977). It can be concluded that the function of excision repair is to minimize the deleterious consequences of DNA damage, such as the persistence of UV photoproducts.

A similar process takes place in humans. Although much less is known about the mechanism, many genes have been shown to be involved, and these are being cloned and the role of their products is being elucidated (Hoeijmakers & Bootsma, 1990; Bootsma & Hoeijmakers, 1991). Like bacteria, humans can also be deficient in aspects of excision repair. The prototypic example is the genetic disorder xeroderma pigmentosum, which is actually a complex of disorders comprising at least 10 different forms of DNA repair defect (nine excision defective complementation groups and one excision repair proficient variant group) (Kraemer *et al.*, 1987; Cleaver & Kraemer, 1989). The sensitivity of fibroblasts and lymphocytes from excision-defective individuals with xeroderma pigmentosum to mutation and lethality by UVC is up to 10 times greater than that of cells from normal individuals (Arlett *et al.*, 1992) and for UVR from a solar simulator (Patton *et al.*, 1984). The pigmentary abnormalities are confined to sun-exposed portions of the skin.

The incidences of tumours of the skin, anterior eye and tip of the tongue in these individuals are much higher than those in unaffected populations (Kraemer *et al.*, 1987), and the median age of patients at onset of skin cancers appears to be much younger than that of the general population. Multiple primary skin cancers are common, which arise predominantly on sunlight-exposed areas of the body (Kraemer *et al.*, 1987); there is anecdotal information that they are largely prevented if protection against exposure to sunlight is afforded early in life (Kraemer & Slor, 1984). Studies of patients with excision-defective xeroderma pigmentosum provide the strongest evidence that sunlight-induced photoproducts can result (in the absence of repair) in the genesis of basal-cell carcinomas, squamous-cell carcinomas and melanomas and strongly support the contention that they can also do so in normal individuals in whom repair is more efficient (although probably never complete). The photoproducts that fail to be excised in xeroderma patients are known to be produced in human skin, not only by UVC (used in most laboratory experiments with cells) but also by UVB, particularly by wavelengths around 300 nm (Bridges, 1990; Athas *et al.*, 1991). Action spectra show that the difference in the cytotoxic action of UVB on cultured cells from normal and xeroderma pigmentosum patients is similar to that of UVC, whereas the differences in the response to UVA are only slight (Keyse *et al.*, 1983). The studies on xeroderma pigmentosum illustrate that DNA repair is a major defence of the human skin against the carcinogenic action of sunlight.

4.4.2 *Trichothiodystrophy*

The conclusions derived from studies of xeroderma pigmentosum have become more complex with the availability of information on two related excision disorders. Trichothiodystrophy is a rare disease in which patients generally have skin judged to be sun-sensitive by erythemal response but no indication of the pronounced freckling or elevated incidence of

early skin tumours associated with xeroderma pigmentosum (Bridges, 1990). In the majority of cases studied, trichothiodystrophy is associated with a deficiency in the ability to repair UV-induced damage in cellular DNA.

Three categories of response to UVR have been identified. In type 1, the response is completely normal, whereas type-2 cells are deficient in excision repair, with properties indistinguishable from those of xeroderma pigmentosum complementation group D. Type-3 cells survive normally after UV irradiation, and the rates of removal of cyclobutane pyrimidine dimer sites are also normal (Broughton *et al.*, 1990). In xeroderma pigmentosum diploid fibroblast lines, catalase activity was decreased on average by a factor of five as compared to controls, while heterozygotic lines exhibited intermediary responses. All trichothiodystrophy lines tested were deficient in UV-induced lesion repair and exhibited a high level of catalase activity; however, molecular analysis of catalase transcription showed no difference between normal, xeroderma and trichothiodystrophy cell lines. UV irradiation induces five times more hydrogen peroxide production in xeroderma lines than in trichothiodystrophy lines and three times more than in controls. These striking differences indicate that UVR, directly or indirectly, together with defective oxidative metabolism may increase the initiation and/or the progression steps in patients with xeroderma pigmentosum to a greater degree than in people with trichothiodystrophy, which may partly explain the different tumoral phenotypes in the two diseases (Vuillaume *et al.*, 1992).

Five patients with trichothiodystrophy type 2 appeared to be in one of the xeroderma pigmentosum complementation groups: Fibroblasts from these individuals were indistinguishable from xeroderma fibroblasts in the same complementation group and were equally sensitive to the lethal and mutagenic effects of UVC (Stefanini *et al.*, 1986; Lehmann *et al.*, 1988). Two other trichothiodystrophy patients (type 3) had cells markedly defective in the removal of (6-4) pyrimidine photoproducts but not cyclobutane-type dimers (Broughton *et al.*, 1990).

4.4.3 *Cockayne's syndrome*

A third sun-sensitive excision repair disorder is Cockayne's syndrome. Patients with this condition have fibroblasts which undergo normal excision repair in the overall genome but which are defective in the excision of dimers from DNA strands undergoing active transcription (Mayne *et al.*, 1988). Cockayne's syndrome cells are sensitive to both killing and mutation induction by UVC (Arlett & Harcourt, 1983) and have reduced repair of cyclobutane dimers; they show, however, normal repair of non-dimer photoproducts in a UV-treated shuttle vector plasmid. Like patients with trichothiodystrophy, those with Cockayne's syndrome do not have pronounced freckling or enhanced early incidence of skin cancers (Barrett *et al.*, 1991).

4.4.4 *Role of immunosuppression*

If it is assumed that UV-induced DNA damage sustained by patients with trichothiodystrophy type 2 results in the same photo-induced mutations in their skin cells (including mutations associated with the initiation of cancer) as is seen in xeroderma pigmentosum patients of the same complementation group (D) (Bridges, 1990; Broughton *et al.*, 1990), something other than unrepaired DNA damage and an elevated frequency of mutations must be needed to trigger initiated cells into clonal expansion and early tumours, as is seen in

xeroderma pigmentosum. The assumed latency of initiated cells in such trichothiodystrophy patients may be related to the latency seen in epidemiological studies of skin cancer in the normal population (see section 2).

The nature of the circumstances that allow initiated skin cells to develop into tumours in xeroderma pigmentosum patients, and perhaps later in life in other individuals, is unclear. Burnet (1971) first suggested that individuals with this disorder might be deficient in some immunosurveillance step. Bridges (1990) proposed that they were also hypersensitive to both the immunosuppressive and the mutagenic action of UVR, so that the elevated skin cancer rate in individuals with xeroderma pigmentosum would not accurately reflect the actual increase in mutation frequency in exposed skin but would exaggerate it greatly.

4.5 Genetic and related effects

Any cell that is UV-irradiated can be expected to sustain DNA damage. The nature of this damage is wavelength-dependent, and the major photoproducts of short-wavelength UV irradiation are various types of dipyrimidine photoproducts, while DNA strand breakage and DNA–protein cross-linkage occur relatively more frequently after irradiation with long-wavelength UVR. As the wavelength is increased above 290 nm, the efficiency of formation of pyrimidine dimers and other DNA photoproducts decreases greatly. This wavelength-dependency of response presents a fundamental problem for the quantitative interpretation of the genetic activities of different regions of the UV spectrum. In most experimental studies with UVA and UVB irradiation and, of course, simulated solar radiation, monochromatic radiation was not used. Also, the characteristics of the radiation emitted from the source are variable over time and from source to source. Because of these practical considerations, comparisons of the effects seen in different studies in terms of dose are commonly invalid: Photoproduct yield is dependent on the energy contributions from the different wavelengths within the spectrum used, but incident doses (fluences) are measured only as energy fluxes over the whole spectrum emitted from the source. The problem of dosimetry within experimental systems is compounded by the fact that absorbed dose is determined by the geometry of the system and the position of the target within it: absorption by one layer (e.g., the medium or a layer of cells) will affect the fluence incident upon the layer beneath. The fluence absorbed may thus differ substantially from the incident fluence of the system. For these reasons, it was considered inappropriate to compile quantitative genetic profiles as is customary in these monographs.

Given the generally significant responses in many different tests for the genetic activity of UVR in a wide range of organisms and cultured cells, the simple qualitative questions appear to have been answered in abundance. The main issues of outstanding interest are: identification of the types of damage induced by the various portions of the UV spectrum; the mechanisms by which damage is translated into mutation or other genetic changes; and the dose characteristics of these responses.

4.5.1 *Humans*

The portions of the body that receive most exposure to UVR are the skin, anterior eye and lip. Because dermal capillaries approach the skin surface, it can be anticipated that blood

will be exposed to the portion of UVR (see Kraemer & Weinstein, 1977; Morison *et al.*, 1979a; Larcom *et al.*, 1991) that penetrates the dermis. The biological consequences of this exposure are unknown.

DNA damage in skin cells has been studied using three methods that are sensitive enough to detect DNA damage after exposure to doses of UVR too low to induce erythema:

(i) use of antibodies specific for UV-altered DNA, followed by immunofluorescence. This method can be used with immunoperoxidase staining and a secondary antibody (Eggset *et al.*, 1983, 1986) or without them (Tan & Stoughton, 1969);

(ii) autoradiography after tritiated thymidine incorporation (Epstein *et al.*, 1969, 1970; Hönigsmann *et al.*, 1987; Wolf *et al.*, 1988); and

(iii) treatment of extracted DNA with *Micrococcus luteus* cyclobutyl pyrimidine dimer site-specific endonuclease, followed by alkaline agarose gel electrophoresis of the single-stranded DNA fragmented at the dimer sites (Sutherland *et al.*, 1980; D'Ambrosio *et al.*, 1981; Gange *et al.*, 1985; Freeman *et al.*, 1986, 1987, 1989; Alcalay *et al.*, 1990). This method suffers the disadvantage that damage cannot be localized to particular layers of the skin, but dimer yield can be calculated. Methods for the study of resolved genetic damage have not been pursued.

(*a*) *Epidermis*

(i) *Broad-spectrum ultraviolet radiation, including solar simulation*

Effects on DNA synthesis were demonstrated in human skin *in vivo* which had been exposed to three times the MED of UVR (< 320 nm; mercury arc lamp [Fig. 9a, p. 64]) and then injected intradermally with tritiated thymidine (8–41 $\times$ 10^6 ergs/cm^2 [8–41 kJ/m^2]) in the irradiated area immediately and at 0.25, 3, 5 and 24 h subsequently. S Phase was suppressed in cells of the basal layer at 3-h and 5-h sampling times, but not at 24 h. Sparsely labelled cells (indicating DNA repair) occurred in greatly variable proportions from person to person in the basal, malpighian and granular layers at 0, 0.25, 3 and 5 h, but not at 24 h, indicating that repair was complete by 24 h (Epstein *et al.*, 1969). DNA repair was also reduced in the skin cells of three patients with xeroderma pigmentosum in comparison to eight normal controls (Epstein *et al.*, 1970).

Sutherland *et al.* (1980) demonstrated a dose-related response for the induction of pyrimidine dimers after exposure to a Westinghouse sun lamp (Fig. 9c, p. 64), with 50% energy < 320 nm, at 0, 970, 1940 and 3880 J/m^2. In one subject, 0.5 of the MED of sun-lamp exposure resulted in about 6 $\pm$ 0.6 dimers per 10^8 Da.

D'Ambrosio *et al.* (1981) reported that approximately 12.8 and 23.6 dimers per 10^8 Da were induced in skin DNA *in vivo* following irradiation with a mercury arc lamp (200–450 nm) at 150 and 300 J/m^2, respectively. Repair or removal of dimers was measured 0–24 h following exposure. About 50% of the dimers were lost 58 min after irradiation, and less than 10% remained at 24 h. In an experiment with patients with lupus erythematosus, D'Ambrosio *et al.* (1983) obtained results similar to those found in the skin of normal individuals.

Strickland *et al.* (1988) measured the induction of cyclobutane dithymidine photoproducts in human skin samples after exposure to simulated solar radiation. Tissue samples from three non-pigmented (white) individuals were exposed to 18 or 36 kJ/m^2 UVR (0.5–1 MED), and those from three constitutively pigmented (black) individuals were exposed to 72

and 144 kJ/m^2. Constitutively pigmented skin required doses of UVR two to four times higher than non-pigmented skin to produce roughly equivalent levels of thymine dimers. [The Working Group noted the small number of people studied.]

(ii) *UVA radiation*

Freeman *et al.* (1987) showed in two subjects that similar pyrimidine dimer yields were produced in skin by a broad-band UVA source (UVASUN 2000), by broadband UVA filtered to remove all light of wavelengths < 340 nm and by narrow-band radiation centred at 365 nm (xenon–mercury compact arc), indicating that UVA radiation and not stray shorter wavelength radiation was responsible. Dimer production was observed following exposures to 5×10^5 J/m^2. Since exposure to a UVA-emitting tanning lamp results in a dose of about 5×10^5 J/m^2, UVA exposure for cosmetic purposes could result in measurable levels of DNA damage.

(iii) *UVB radiation*

The efficiency of UVA- and UVB-induced tans in protecting against erythema and the formation of dimers induced by UVB was studied in five subjects by Gange *et al.* (1985). The radiation sources were a UVASUN 2000 lamp (UVA; Fig. 8d, p. 61) and an FS36 Elder fluorescent sunlamp (UVB). UVB-induced tanning protected against erythema produced by subsequent UVB exposure two to three times better than UVA-induced tanning; however, tanning with either UVA or UVB was associated with a similar reduction in yield of endonuclease-sensitive sites in epidermal DNA (about 50%).

Eggset *et al.* (1983) observed DNA damage in both epidermis and dermis following exposure to a Westinghouse FS-20 sunlamp (Fig. 9c, p. 64) at 0.5–2 MED (2 MED, 900 J/m^2). The outer layers were more heavily damaged after small doses than the basal layer, which may be better protected by its deeper location and shielding by melanin. The authors claimed that DNA repair was well under way after 4–5 h and was apparently nearly complete at 24 h, as judged by immunofluorescence and immunoperoxidase staining. Repair was faster in the presence of visible light than when irradiated skin was shielded with thick black plastic. [The Working Group noted the absence of quantitative data.]

In a study of two volunteers (Eggset *et al.*, 1986), tanning was shown to protect against DNA damage in skin (induced in a UVB solarium), but the conclusions were based solely on observations of immunofluorescence. [The Working Group noted the absence of quantitative data.]

Freeman *et al.* (1986) measured UVB-induced DNA damage in the skin of seven individuals with different sensitivities to UVB irradiation, as measured by the MED, with irradiation from an FS36 Elder fluorescent sunlamp (280–320 nm). The production of dimers was correlated inversely with the MED. The slopes of the dose–response curves for the most UVB-sensitive individual (MED, 240 J/m^2) and for the least sensitive individual (MED, 1460 J/m^2) were 11.5×10^{-4} and 2.6×10^{-4} dimer sites per 1000 bases per mJ/cm^2 [10 J/m^2], respectively.

Hönigsmann *et al.* (1987) studied unscheduled DNA synthesis in epidermal cells in the skin of 25 male volunteers (four with skin type II and 21 with skin type III; see pp. 168–169) after exposure to doses of UVB of 0.06–6 MED, from a 6-kW xenon arc lamp (292–304 nm). The MED values ranged from 140 to 550 J/m^2. The dose–response curve showed a significant

increase in unscheduled DNA synthesis between 0.06 and 1 MED but no difference between 1 and 6 MED, suggesting a saturation of excision repair *in vivo*.

Freeman (1988) studied interindividual variability in 17 healthy volunteers in the repair of pyrimidine dimers induced following exposure to 0.25–1.5 MED from a Westinghouse FS-40 sunlamp (see Fig. 9c, p. 64). Removal of dimers was detected within 6 h of irradiation. The average half-time for removal of dimers was 11.0 ± 4.3 (SD) h (range, 5.5–21.1 h). [The Working Group noted that the spectra and doses used in this study were different from those used by D'Ambrosio *et al.* (1981). It is not clear if the interindividual variability is greater than the experimental error.]

Interindividual variability in the repair of UVB-induced pyrimidine dimers was also studied by Alcalay *et al.* (1990) in 22 patients aged 31–84 with at least one basal-cell carcinoma. The control group consisted of 19 cancer-free volunteers aged 25–61. Both groups were given one MED of radiation from a 150-W xenon arc solar UV-simulated lamp equipped with a 50-cm liquid light guide and a filter eliminating wavelengths below 295 nm. Dimers were measured immediately and after 6 h. The two groups were similar at time 0, but after 6 h, 22 ± 4% (range about 8–64) of the dimers were removed in the cancer group compared to 33 ± 4% (range about 4–64) in the control group. Of the cancer patients, 23% had repaired more than 30% of the DNA damage, compared to 53% of the control group. [The Working Group noted that it is not clear if the interindividual variability is greater than the experimental error.]

Wolf *et al.* (1988) observed measurable amounts of unscheduled DNA synthesis in the skin of 23 volunteers exposed to 0.5 MED UVB irradiation from a high-pressure mercury lamp [spectral emission not given]. Administration of carotenoids (to reduce light sensitivity in patients with erythropoietic protoporphyria) at a dose of 150 mg per day for 30 days did not significantly alter the amount of unscheduled DNA synthesis (6 ± 1.2 grains/cell before and 8 ± 2 grains/cell after carotenoid treatment; seven subjects). The same investigation showed no significant protection by carotenoids against UVA-, UVB- or PUVA-induced erythema, on the basis of pre- and post-carotenoid MED or minimal phototoxic dose.

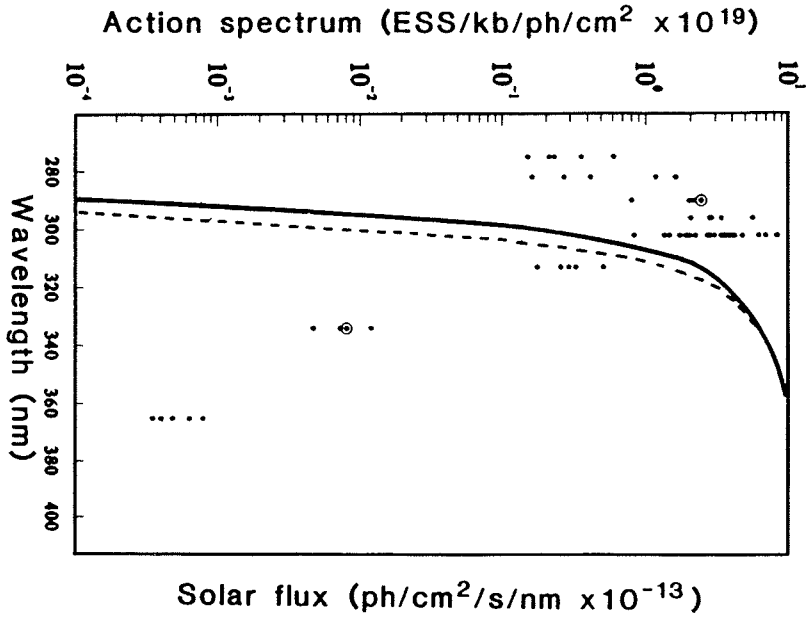
In 30 volunteers, it was demonstrated that the action spectrum for the frequency of pyrimidine dimer formation in human skin DNA for a given fluence (incident dose) has its maximum near 300 nm and decreases sharply on either side of this wavelength (Fig. 12). The decrease at < 300 nm is probably due to absorption in the upper layers of skin. These data were used to estimate that, at a solar angle of 40 °, a reduction in the thickness of the stratospheric ozone layer from 0.32 cm down to 0.16 cm would be expected to result in a 2.5-fold increase in dimer formation (Freeman *et al.*, 1989).

A dose–response for the formation of thymine dimers in epidermal cells isolated from human skin irradiated with UVB *in vitro* was determined by Roza *et al.* (1988) using a monoclonal antibody.

(iv) *UVC radiation*

Exposure of human skin, from which the stratum corneum had been removed, to either a germicidal (UVC) or a Hanovia hot quartz lamp *in vivo* resulted in DNA damage demonstrable by immunofluorescence (Tan & Stoughton, 1969). When the stratum corneum was intact, DNA damage was detected only after exposure to the germicidal lamp. [The

Fig. 12. Action spectrum for pyrimidine dimer formation in human skin (•) and solar spectra at the surface of the Earth for stratospheric ozone levels of 0.32 cm (dotted line) and 0.16 cm (solid line). Each point in the action spectrum represents the slope of the dose–response line (dimer yields at three exposures) for one volunteer at one wavelength, obtained from triplicate independent determinations. Thirty points occur at 302 nm, although some points overlie other values; five points occur at each other wavelength: points at 290 and 334 nm are circled to indicate that identical dimer yields were recorded for two volunteers. ph, photon; ESS, endonuclease-sensitive site



From Freeman *et al.* (1989)

Working Group noted that more sensitive analytical techniques for DNA damage are now available.]

(*b*) *Lymphocytes*

(i) *Broad-spectrum ultraviolet radiation*

In addition to cells of the skin, white blood cells are also subject to exposure to UVB and UVA, partly because some are temporarily resident in the skin and partly because it has been estimated that the equivalent of the total blood volume circulates through the dermal capillaries approximately every 11 min (Kraemer & Weinstein, 1977). Detecting effects, e.g., on lymphocytes, is likely to be extremely difficult owing to the fact that they are continually moving between the blood and other tissues; indeed, 90% of the lymphocyte population at any given time is resident outside the blood. Thus, the concentration in the blood of any

lymphocytes irradiated while passing through the skin may fall substantially over time after irradiation ends as they are diluted in the whole body lymphocyte pool. Extravascular lymphocytes resident in the skin may also receive higher doses of UVR. Nevertheless, studies have been reported of genetic or related effects on lymphocytes sampled from peripheral blood.

Larcom *et al.* (1991) examined the capacity for DNA synthesis of lymphocytes from eight subjects exposed in two commercial tanning salons. Blood was taken immediately before tanning and again 24 h after tanning. System I used a sunlamp with a UVB:UVR ratio of 0.02% for 280–300 nm and 1.4% for 300–315 nm; the output of system II (Solana Voltarc lamp) was not indicated. There was a 24–84% (average, 53%) decrease in phytohaemagglutinin-induced DNA synthesis with system I and a 8–58% (average, 30%) decrease with system II.

(ii) *UVA radiation*

Seven of 13 psoriasis patients receiving oral 8-methoxypsoralen and high-intensity, long-wave UVA radiation had reduced leukocyte DNA synthesis; this did not occur in any of 10 controls (Kraemer & Weinstein, 1977). These results indicate that UVA reduces the incorporation of tritiated thymidine in lymphocytes circulating through the skin.

(iii) *UVB radiation*

In normal, fair-skinned subjects given whole-body exposure to 1.5–3 × MED doses of UVB from a sunlamp (280–380 nm), a dose-dependent decrease was seen in the incorporation of tritiated thymidine into DNA following stimulation by photohaemagglutinin; the proportion of circulating lymphocytes was decreased and the proportion of null cells was increased (Morison *et al.*, 1979a).

These studies indicate that leukocytes should be included in any inventory of human cells potentially exposed to solar radiation or artificial UVR.

4.5.2 *Experimental systems* [see Tables 32–35, in which exposures are separated according to type of UVR]

(*a*) *DNA damage*

Inhibition of DNA synthesis has been induced in hairless albino mouse epidermis at wavelengths of 260–320 nm, with a maximal effect at 290 nm. Inhibition was not detected at 335 nm (Kaidbey, 1988). The action spectrum was similar to that for formation of cyclobutane-type pyrimidine dimers (Cooke & Johnson, 1978; Ley *et al.*, 1983) and pyrimidine–pyrimidone (6-4) photoproducts in mouse skin (Olsen *et al.*, 1989). Pyrimidine dimers (measured as endonuclease-sensitive sites) have been measured in the corneal DNA of the marsupial, *M. domestica*, following exposure to a sunlamp (280–400 nm) (Ley *et al.*, 1988).

While DNA is the main photochromophore for UVC, there is evidence that active oxygen intermediates are involved in the production of DNA damage by UVA (Tyrrell, 1991). The production of several types of photolesions is oxygen dependent (Tyrrell, 1984, 1991). In addition, the irradiation lethality of both cultured bacterial (Webb, 1977) and mammalian (Danpure & Tyrrell, 1976) cells is dependent on the presence of oxygen; this observation was later linked with the production of singlet oxygen (Tyrrell & Pidoux, 1989). It has also been

observed that irradiation of cultured human skin cells with UVB (302 nm, 313 nm), UVA (334 nm, 365 nm) and visible (405 nm) radiation is strongly enhanced in glutathione-depleted cells (Tyrrell & Pidoux, 1986, 1988). This apparent protection by glutathione appears to be due to its radical scavanging properties at the stated wavelength but may be due to induction of a more specific pathway (such as its essential role as a hydrogen donor for glutathione peroxidase) at longer wavelengths. Francis and Giannelli (1991) found that the abnormally high yield of single-stranded DNA breaks produced by UVA in six UVA-sensitive human fibroblasts (three from actinic reticuloid patients, two from sisters with familial actinic keratoses and internal malignancies and one from a patient with an abnormally high incidence of basal-cell carcinomas) could be reduced if sensitive cells were co-cultivated with normal fibroblasts or with radical scavengers. They suggested that the UVA-sensitive cells had deficits of small-molecular-weight scavengers of active oxygen species and that intercellular cooperation allows the transfer of these substances from resistant to sensitive cells. The presence of non-DNA chromophores that generate active oxygen species can also occur with UVC. Melanin, normally regarded as a solar screen, has also been associated with the formation of oxidative DNA damage, such as thymine glycols in mouse cells that vary in melanin content (Huselton & Hill, 1990). A slight increase in pyrimidine dimer yield was seen in human melanocytes as compared to keratinocytes following exposure to UVR at 254, 297, 302 and 312 nm but was significant only at 297 nm (Schothorst *et al.*, 1991).

(*b*) *Mutagenicity*

Numerous reports show that sunlight or solar-simulated radiation induces mutations in bacteria, plants, Chinese hamster ovary (CHO) and lung (V79) cells, mouse lymphoma cells and human skin fibroblasts.

Studies in bacteria exposed to radiation throughout the solar UV spectrum (reviewed by Webb, 1977) demonstrate mutagenic activity unambiguously. The effects of sunlight on mammalian cells have been reviewed (Kantor, 1985). UVA (320–400 nm) is mutagenic to yeast and cultured mammalian cells, UVB (290–320 nm) to bacteria and cultured mammalian cells and UVC (200–290 nm) to bacteria, fungi, plants, cultured mammalian cells, including CHO and V79 cells, and human lymphoblasts, lymphocytes and fibroblasts. Since wavelengths in the UVC range do not reach the surface of the Earth, they are of no significance as a source of damage in natural sunlight.

A characteristic of all of these studies is that UVA appears to be relatively inefficient as a mutagen in comparison with UVB and UVC when activity is expressed per unit of energy fluence, but not necessarily so when expressed per DNA photoproduct (see Tyrrell, 1984). Webb (1977) compiled action spectra for the introduction of mutations in bacteria, as did Coohill *et al.* (1987) for mutagenesis in human epithelial cells. In both *Salmonella* and human cells, wavelengths > 320 nm were at least 10^3 times less effective than those between 270 and 290 nm.

A comparison of the mutagenicity of various UV-containing light sources towards a set of *S. typhimurium* strains was reported by De Flora *et al.* (1990). The approach did not involve measurement of cytotoxicity, and mutagenicity was compared at roughly equitoxic doses rather than as a function of fluence. Halogen lamps were as mutagenic as 254-nm UVC and more mutagenic than fluorescent sunlamps or sunlight. The mutagenicity of halogen lamps

was attributed to their UVC component, in contrast to sunlight which produced mutagenic effects over a wide UV spectrum. The mutagenicity of halogen lamps, fluorescent lamps and sunlight was partially inhibited by catalase, suggesting that peroxides may be involved in this in-vitro system. It is also relevant that pretreatment of *E. coli* with hydrogen peroxide results in an increase in both UVA resistance and hydrogen peroxide scavenging ability (Moss, S.H., quoted by Tyrrell, 1985; Sammartano & Tuveson, 1985; Tyrrell, 1985).

Further evidence for the complexity of responses to the UVR region comes from Schothorst *et al.* (1987b), who examined the mutational response of human skin fibroblasts to 12 lamps differing widely in their emission characteristics. Surprisingly, they found that, whatever the light source, mutation induction per MED was similar with UVC, UVB and solar radiation; with UVA (only one data point), mutation induction per MED was much greater. The authors emphasized that these conclusions hold only if it is valid to calculate the mutagenicity of a light source by adding the effects of the contributing wavelengths; however, the data of Coohill *et al.* (1987) argue against this assumption.

The inevitable consequence of the absorption spectrum maximum of DNA is that there is a considerable body of data on mutagenicity toward microorganisms of UVC, which is usually delivered by radiation from germicidal lamps with more than 90% of their output at 254 nm. The types of mutations that are induced by UVC and the mechanisms of their induction have been reviewed (Witkin, 1976; Hall & Mount, 1981; Walker, 1984; Hutchinson & Wood, 1986; Bridges *et al.*, 1987; Hutchinson, 1987). Specific cellular proteins, including the products of *recA* and *umuC* genes, together with a cleaved derivative of the *umuD* gene product, must be present for mutations to result from most types of DNA damage. These proteins are themselves part of an inducible response to DNA damage, and their intracellular level increases dramatically when photoproducts or other lesions are detected in DNA. It is not yet clear to what extent inducible systems are involved in UV mutagenesis in higher eukaryotes.

Current evidence suggests that all photoproducts are likely to be potentially mutagenic, although with greatly different specificities and potencies. The major UV photoproducts, cyclobutane-type thymine–thymine dimers, are, for example, relatively weakly mutagenic (Banerjee *et al.*, 1988, 1990), owing in part to the propensity of polymerases to insert adenine when the template instruction is unclear or missing (Sagher & Strauss, 1983; Schaaper *et al.*, 1983; Kunkel, 1984). The relatively minor (6-4) thymine–thymine photoproduct is, in contrast, highly mutagenic, the dominant mutation being a 3′ T→C transition (LeClerc *et al.*, 1991). By far the most frequent UVC-induced change in human cells is the transition from G:C to A:T (Bredberg *et al.*, 1986; Seetharam *et al.*, 1987; Hsia *et al.*, 1989; Dorado *et al.*, 1991). A number of investigators have noted the production of tandem transitions from G:C,G:C to A:T,A:T. Although this is not the most frequent change, it seems to be particularly characteristic for UVC mutagenesis in human cells. The frequency of mutation per lethal event at the *hprt* locus (which detects a broad spectrum of mutations) is approximately the same at 254 nm and 313 nm in human lymphoblastoid cells; however, the mutation frequency per lethal event at the Na^+/K^+ ATPase locus (which detects point mutations) is considerably higher at 313 nm. This finding may indicate a difference in types of premutagenic lesions and/or rates of mutation between the two wavelength regions (Tyrrell, 1984).

Two bacterial studies provide positive evidence for the mutagenic activity of fluorescent lamps. De Flora *et al.* (1990) employed Sylvania 36 W cool white tubes with *E. coli* and *Salmonella* strains. [The Working Group had difficulty in evaluating these data because they are presented in a highly transformed format.] Hartman *et al.* (1991) used General Electric F15T8CW lamps; a lowest effective dose of 5500 J/m^2 can be estimated from the results with *Salmonella* tester strains. Filters that block wavelengths < 370 nm effectively eliminated mutagenesis, while radical scavengers such as superoxide dismutase or catalase stimulated mutagenesis.

Hsie *et al.* (1977) irradiated the *hprt* CHO system with Westinghouse white light F40CW lamps. The minimal effective dose was 3.96×10^6 J/m^2. Putting lids on the petri dishes reduced mutant frequency by 30%. [The Working Group noted that the results were based on a single dose point in a single experiment.] Jacobson *et al.* (1978) exposed mouse lymphoma L5178Y $tk^{+/-}$ cells to Sylvania F18T8 cool white lamps. The estimated lowest effective dose was 2×10^4 J/m^2. [The Working Group noted that the selective agent used, BUdR, is regarded as inefficient and has been superseded by trichlorothymidine, so these results require confirmation.]

(*c*) *Chromosomal effects*

Sunlamps have been shown to produce sister chromatid exchange in amphibian cells (Chao & Rosenstein, 1985) and in human fibroblasts (Bielfeld *et al.*, 1989; Roser *et al.*, 1989). Fibroblasts from a panel of cutaneous malignant melanoma patients (Roser *et al.*, 1989) and heterozygotes of xeroderma pigmentosum (Bielfeld *et al.*, 1989) were more susceptible to the induction of both sister chromatid exchange and micronuclei than those from normal donors. Micronuclei were also induced in mouse splenocytes by exposure to sunlamps *in vitro* (Dreosti *et al.*, 1990).

A study with CHO cells provided evidence for a dose-related increase in the induction of sister chromatid exchange by UVA, but the increased induction of chromosomal aberrations showed no dose–response relationship (Lundgren & Wulf, 1988).

UVB induced sister chromatid exchange in CHO cells (Rasmussen *et al.*, 1989) and chromosomal aberrations in frog ICR 2A cells (Rosenstein & Rosenstein, 1985). In the latter study, photoreactivation reduced the number of chromosomal aberrations more effectively at 265, 289 and 302 than at 313 nm, suggesting that non-cyclobutane dimer photoproducts are more important primary lesions at the higher wavelength.

For UVC, more extensive data are available. Sister chromatid exchange was induced in Chinese hamster V79 (Nishi *et al.*, 1984) and CHO (Rasmussen *et al.*, 1989) cells. Chromatid exchange was also recorded in cultured fetal fibroblasts from New Zealand black mice, which proved to be more sensitive than BALB/c cells (Reddy *et al.*, 1978). The induction of chromosomal aberrations in Chinese hamster cells has been reported on a number of occasions (Chu, 1965a,b; Trosko & Brewen, 1967; Bender *et al.*, 1973; Griggs & Bender, 1973; Ikushima & Wolff, 1974).

Exposure of frog ICR 2A cells to 254 or 265 nm radiation induced both sister chromatid exchange (Chao & Rosenstein, 1985) and chromosomal aberrations, while photoreactivating light significantly reduced the frequency of chromosomal aberrations, which implies a role for pyrimidine dimers in their genesis (Rosenstein & Rosenstein, 1985). Chromosomal

aberrations were also seen with *Xenopus* cell cultures (Griggs & Bender, 1973). The frequencies of sister chromatid exchange and chromosomal aberrations induced by UVC were reduced by photoreactivating light in chicken embryo fibroblasts (Natarajan *et al.*, 1980), lending further support to the concept that the cyclobutane pyrimidine dimer represents a primary lesion in these two end-points.

Parshad *et al.* (1980a) reported the induction of chromosomal damage in human IMR-90 fibroblasts following treatment with 4.6 W/m^2 over 20 h (331 kJ/m^2) from F15T8-CW tubes. Shielding and radical scavengers reduced the level of damage.

Extensive data are available on the induction of sister chromatid exchange in fibroblasts from patients with Bloom's syndrome (Krepinsky *et al.*, 1980), xeroderma pigmentosum (De Weerd-Kastelein *et al.*, 1977; Fujiwara *et al.*, 1981) or Cockayne's syndrome (Marshall *et al.*, 1980; Fujiwara *et al.*, 1981), as well as from normal individuals. In comparison with normal individuals, more sister chromatid exchanges were induced per lethal lesion in fibroblasts from excision-competent Bloom's syndrome (Kurihara *et al.*, 1987) and Cockayne's syndrome (Marshall *et al.*, 1980) patients. No such increase in sister chromatid exchange was seen in fibroblasts from excision-defective xeroderma pigmentosum patients or from an individual defective in the ligation step of repair (Henderson *et al.*, 1985).

The induction of sister chromatid exchange by UV irradiation has also been studied in human lymphocytes, with conflicting results. In one study, they were reported to be less responsive than either human fibroblasts or CHO cells (Perticone *et al.*, 1986), while another report, in which chromosomal aberrations were also studied, suggested that lymphocytes were more sensitive than fibroblasts in their response at both end-points (Murthy *et al.*, 1982). These results may have implications for the interpretation of the effect of UV on the immune system.

Fibroblasts from xeroderma pigmentosum patients are more sensitive to the induction of chromosomal aberrations than cells from normal donors (Parrington *et al.*, 1971; Parrington, 1972; Marshall & Scott, 1976). Seguin *et al.* (1988) showed that lymphoblastoid cells from five Cockayne's syndrome patients were similarly hypersensitive to UVC-induced chromosomal aberrations. The induction of micronuclei in two normal and three Bloom's syndrome-derived fibroblast cell cultures was reported by Krepinsky *et al.* (1980). One culture from a Bloom's syndrome patient, GM1492, proved to be exceptionally sensitive to the induction of micronuclei; the other two were indistinguishable from normal cells. This result emphasizes the potential importance of heterogeneity in response among patients with rare genetic syndromes.

(*d*) *Transformation*

Morphological transformation of mammalian cells has been induced by solar radiation, unshielded fluorescent tubes, solar simulators, UVA, UVB and, most extensively, UVC. There is weak evidence (Baturay *et al.*, 1985) for the induction of transformation by predominantly UVA radiation (20T12BLB bulbs) in BALB/c 3T3 cells. In the same report, UVA was shown to have promoting activity following initiation with β-propiolactone. The most effective wavelength for Syrian hamster embryo cells (Doniger *et al.*, 1981) and human embryonic fibroblasts (Sutherland *et al.*, 1981) appears to be in the UVC range at about 265 nm. Transformation of human cells can be enhanced by delivering the dose on a number of

separate occasions (Sutherland *et al.*, 1988). It has also been reported that excision repair-defective xeroderma pigmentosum cells can be transformed to the anchorage-independent phenotype at lower doses than those required for cells from normal individuals (Maher *et al.*, 1982). Fisher and Cifone (1981) showed enhanced metastatic potential of mouse fibrosarcoma cells. Plasmids containing the human N-*ras* gene which were irradiated with UVR (254 nm) *in vitro* acquired the ability to transform cultured rat-2 cells after transfection; photoreactivation of irradiated plasmids eliminated their transforming ability (van der Lubbe *et al.*, 1988). In another study, UVB irradiation activated the human Ha-*ras* gene on a plasmid in a transformation assay with mouse NIH-3T3 cells (Pierceall & Ananthaswamy (1991).

An investigation of chromosomal breaks and malignant transformation in embryonic mouse cells (Sanford *et al.*, 1979; Parshad *et al.*, 1980b) revealed that exposure of cultured cells to fluorescent lamps induced malignant transformation, as measured by tumour formation following implantation into syngeneic hosts. The potential importance of active oxygen species was revealed by experiments in which the partial pressure of oxygen in cultures was increased, resulting in increased malignant transformation and correlated chromosomal breakage.

Kennedy *et al.* (1980) reported induction of transformation in C3H 10T½ mouse embryonic cell cultures by light from General Electric F18T8 lamps. The lowest effective dose was estimated at 2×10^5 J/m^2, and use of petri dish lids was effective in reducing transformation.

(*e*) *Effects on cellular and viral gene expression*

A number of cellular oncogenes and other genes involved in the regulation of growth are implicated in the process of carcinogenesis, as they are subject to both gene mutation and alteration in expression due to chromosomal rearrangement. Many of these genes also show transient alterations in expression following DNA damage, which has led to the suspicion that such transient changes are involved, either directly or indirectly, in the carcinogenic process.

UVC radiation was found to increase transiently the expression of various cellular genes, including those that code for collagenase (Stein *et al.*, 1989), the fos protein (Hollander & Fornace, 1989; Stein *et al.*, 1989), the jun protein (Ronai *et al.*, 1990), metallothioneins I and II (Fornace *et al.*, 1988) and human plasminogen activator (Miskin & Ben-Ishai, 1981). UVA radiation enhanced expression of the genes that code for the fos protein (Hollander & Fornace, 1989), and UVB radiation increased the level of ornithine decarboxylase (Verma *et al.*, 1979). Different levels of cytotoxicity were seen in these experiments. UVA radiation at doses that inactivate a small fraction of the fibroblast cell population induced expression of the haem oxygenase gene (Keyse & Tyrrell, 1989) by a transient enhancement in transcription rate (Keyse *et al.*, 1990). *cis*-Acting enhancer elements have been shown to be involved in activation of the collagenase and c-*fos*, as well as human immunodeficiency promoter (Stein *et al.*, 1989). In both rat fibroblasts and human keratinocyte cell lines, exposure to UVR increased the levels of c-*fos* RNA within 10 min and of c-*myc* RNA after about 1 h. The levels peaked at 30 min and 7 h and returned to normal within 1 h and 24 h, respectively. The order of effectiveness was UVC > UVB > UVA

(Ronai *et al.*, 1990). Elevated levels of p53 protein were observed in mouse cells treated with UVR; the increase was due to post-translation activation or stabilization (Maltzman & Czyzyk, 1984). In human keratinocytes exposed to UVA, increased levels of human epidermal growth factor receptor RNA (HER-1) were found (Yang *et al.*, 1988).

The mechanisms that mediate these transient and immediate inducible responses are largely unknown. Some of them, however, overlap with those seen in response to tumour promoters, and it is significant that natural sunlight has been reported to enhance the expression of protein kinase C in cultured human epithelial P3 cells (Peak *et al.*, 1991a). For reviews of this general area, see Ananthaswamy and Pierceall (1990) and Ronai *et al.* (1990).

Other transient responses to UVR have been noted at somewhat later times (12–48 h). Methotrexate resistance due to gene amplification was reported in 3T6 mouse cells (Tlsty *et al.*, 1984). Another selective DNA amplification response is induction by UVR of viral DNA synthesis, e.g., of polyoma virus in rat fibroblasts. UVC was more effective than UVB, and UVA was ineffective (Ronai *et al.*, 1987). In Chinese hamster embryo cells, UVC irradiation increased DNA binding to the early domain of the SV40 minimal origin, resulting in SV40 DNA amplification (Lücke-Huhle *et al.*, 1989). The induction of asynchronous viral replication is mediated by cellular proteins that bind to specific sequences in the DNA of polyoma (Ronai & Weinstein, 1988) and SV40 viruses (Lücke-Huhle *et al.*, 1989).

Exposure to UVR can activate viruses. This phenomenon has been known for herpes simplex virus for a long time (for a recent report, see Rooney *et al.*, 1991). It was reported recently that UVC can activate the gene promoters of the human immunodeficiency virus (HIV) (Valerie *et al.*, 1988) and Moloney murine sarcoma virus (Lin *et al.*, 1990). Furthermore, activation of complete HIV grown in cells pre-exposed to UVC radiation was observed (Valerie *et al.*, 1988). HIV activation may contribute to faster development of AIDS, which in turn may facilitate development of malignancies. Further studies showed that the HIV promoter and HIV are activated by UVC and UVB, but not UVA radiation even at very high exposures (Stanley *et al.*, 1989; Beer *et al.*, 1991 [abstract]; Lightfoote *et al.*, 1992). There are indications that pyrimidine dimers (Stein *et al.*, 1989) or chromatin damage (Valerie & Rosenberg, 1990) play a role in the initiation of HIV activation by UVR. The in-vitro observations have been verified for UVC, UVB and UVA in experiments with transgenic mice carrying the HIV promoter/reporter gene constructs (Cavard *et al.*, 1990; Frucht *et al.*, 1991; Vogel *et al.*, 1992). For reviews on the activation HIV by UVR, see Zmudzka and Beer (1990) and Beer and Zmudzka (1991).

Table 32. Genetic and related effects of solar, simulated solar and sunlamp (UVA and UVB) irradiation

Test system	Result[a]	Reference
BS?, *Bacillus subtilis*, mutation	+	Munakata (1989)
SSB, *Saccharomyces cerevisiae* D7, DNA damage	+	Hannan *et al.* (1984)
PLM, Wheat mutation	+	Morgun *et al.* (1988)
DIA, DNA damage, ICR 2A frog cells *in vitro*	+	Chao & Rosenstein (1986)
DIA, DNA damage, ICR 2A frog cells *in vitro*	+	Rosenstein *et al.* (1989)
DIA, DNA strand breaks, Chinese hamster V79 cells	+	Elkind & Han (1978)
DIA, DNA damage, Chinese hamster V79 cells *in vitro*	+	Suzuki *et al.* (1981)
DIA, DNA damage, C3H 10T½ mouse cells *in vitro*	+	Suzuki *et al.* (1981)
GCO, Gene mutation, Chinese hamster ovary cells *in vitro*	+	Hsie *et al.* (1977)
G9H, Gene mutation, Chinese hamster V79 lung cells *in vitro*, 6-TG^r	+	Zölzer *et al.* (1988)
G5T, Gene mutation, mouse lymphoma L5178Y cells *in vitro*	+	Jacobson *et al.* (1978)
G9H, Gene mutation, Chinese hamster V79 lung cells *in vitro*, 6-TG^r	+	Bradley & Sharkey (1977)
GCO, Gene mutation, Chinese hamster ovary cells *in vitro*	+	Burki & Lam (1978)
G9H, Gene mutation, Chinese hamster V79 lung cells *in vitro*, 6-TG^r	+	Suzuki *et al.* (1981)
SIA, Sister chromatid exchange, ICR 2A frog cells *in vitro*	+	Chao & Rosenstein (1985)
MIA, Micronucleus test, mouse splenocytes *in vitro*	+	Dreosti *et al.* (1990)
TBM, Cell transformation, BALB/c 3T3 mouse cells *in vitro*	+	Withrow *et al.* (1980)
TBM, Cell transformation, BALB/c mouse epidermal cells *in vitro*	+	Ananthaswamy & Kripke (1981)
TCM, Cell transformation, C3H 10T½ mouse embryo cells *in vitro*	+	Kennedy *et al.* (1980)
TCM, Cell transformation, C3H 10T½ mouse cells *in vitro*	+	Suzuki *et al.* (1981)
TCL, Cell transformation, mouse fibrosarcoma cells *in vitro*	+	Fisher & Cifone (1981)
TCL, Cell transformation, 10T½ mouse skin fibroblasts *in vitro*	+	Ananthaswamy (1984a)
DIA, DNA damage, fish *in vitro*	+	Applegate & Ley (1988)
DIH, DNA damage, human skin fibroblasts *in vitro*	+	Rosenstein *et al.* (1985)
DIH, DNA damage, human skin fibroblasts *in vitro*	+	Chao & Rosenstein (1986)
DIH, DNA damage, human skin fibroblasts *in vitro*	+	Rosenstein (1988)
DIH, DNA damage, human skin fibroblasts *in vitro*	+	Rosenstein & Mitchell (1991)
DIH, DNA damage, human HeLa cells *in vitro*	+	Elkind & Han (1978)
GIH, Gene mutation, human xeroderma pigmentosum fibroblasts *in vitro*	+	Patton *et al.* (1984)
SHF, Sister chromatid exchange, human[b] fibroblasts *in vitro*	+	Knees-Matzen *et al.* (1991)

Table 32 (contd)

Test system	Result[a]	Reference
SIH, Sister chromatid exchange, human xeroderma pigmentosum fibroblasts	+	Bielfeld *et al.* (1989)
SIH, Sister chromatid exchange, human malignant melanoma cells	+	Roser *et al.* (1989)
MIH, Micronucleus test, human xeroderma pigmentosum fibroblasts	+	Bielfeld *et al.* (1989)
MIH, Micronucleus test, human malignant melanoma cells	+	Roser *et al.* (1989)
DVA, DNA damage, BALB/c mouse skin cells *in vivo*	+	Ananthaswamy & Fisher (1981)
DVA, DNA damage, marsupial corneal cells *in vivo*	+	Freeman *et al.* (1988a)
DVA, DNA damage, marsupial corneal cells *in vivo*	+	Ley *et al.* (1988)
TVI, Cell transformation, 10T½ mouse skin fibroblasts treated *in vivo* scored *in vitro*	+	Ananthaswamy (1984b)
DVH, DNA damage, human skin cells *in vivo*	+	Eggset *et al.* (1983)
DVH, DNA damage, human skin cells *in vivo*	+	Freeman *et al.* (1988b)

[a] +, positive
[b] First-degree relatives of melanoma patients

Table 33. Genetic and related effects of predominantly UVA irradiation (near UV)

Test system	Result[a]	Reference
SA9, *Salmonella typhimurium* TA98, reverse mutation	+	Calkins *et al.* (1987)
ECW, *Escherichia coli* WP2 *uvr*A, reverse mutation	+	Tyrrell (1982)
EC2, *Escherichia coli* WP2 hcr–, reverse mutation	+	Kubitschek (1967)
ECR, *Escherichia coli* B/r/l, *trp*, reverse mutation	+	Webb & Malina (1970)
ECR, *Escherichia coli* WP2 *rec*A, reverse mutation	+	Tyrrell (1982)
ECR, *Escherichia coli* WP2 *uvr*A *rec*A, reverse mutation	+	Tyrrell (1982)
ECR, *Escherichia coli* B/r *uvr*A *trp thy*, reverse mutation	+	Tyrrell (1982)
ECR, *Escherichia coli* wild type, reverse mutation	+	Tyrrell (1982)
ECR, *Escherichia coli*, mutation	+	Wood *et al.* (1984)
SSB, *Saccharomyces cerevisiae* wild type, DNA damage	+	Zölzer & Kiefer (1983)
SSB, *Saccharomyces cerevisiae* excision-deficient, DNA damage	+	Zölzer & Kiefer (1983)
SSB, *Saccharomyces cerevisiae* D7, DNA damage	+	Hannan *et al.* (1984)
DIA, DNA damage, Chinese hamster ovary cells *in vitro*	+	Zelle *et al.* (1980)
DIA, DNA strand breaks, Chinese hamster ovary cells *in vitro*	+	Churchill *et al.* (1991)
GCO, Gene mutation, Chinese hamster ovary cells *in vitro*	+	Zelle *et al.* (1980)
GCO, Gene mutation, Chinese hamster ovary cells *in vitro*	+	Singh & Gupta (1982)
GCO, Gene mutation, Chinese hamster ovary cells *in vitro*	+	Lundgren & Wulf (1988)
G9H, Gene mutation, Chinese hamster lung V79 cells, *hprt* locus	+	Wells & Han (1984)
G9O, Gene mutation, Chinese hamster lung V79 cells, 6-TG^r	+	Wells & Han (1984)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus	+	Hitchins *et al.* (1987)
SIC, Sister chromatid exchange, Chinese hamster ovary cells *in vitro*	+	Lundgren & Wulf (1988)
CIC, Chromosomal aberrations, Chinese hamster ovary cells *in vitro*	(+)	Lundgren & Wulf (1988)
TCL, Cell transformation, Syrian hamster embryo cells *in vitro* (neoplastic transformation)	+	Barrett *et al.* (1978)
TCL, Cell transformation, Syrian hamster embryo cells *in vitro* (morphological transformation)	-	Barrett *et al.* (1978)
DIH, DNA strand breaks, human fibroblasts *in vitro*	+	Rosenstein & Ducore (1983)
DIH, DNA strand breaks, human teratoma cells *in vitro*	+	Peak *et al.* (1987)
DIH, DNA double strand breaks, human teratocarcinoma cells *in vitro*	+	Peak & Peak (1990)
DIH, DNA strand breaks, human fibroblasts *in vitro*	+	Francis & Giannelli (1991)
DIH, DNA-protein cross-links, human teratocarcinoma cells *in vitro*	+	Peak & Peak (1991)
DIH, DNA strand breaks, human epithelial P3 cells *in vitro*	+	Peak *et al.* (1991b)
DIH, Pyrimidine dimer formation, human skin fibroblasts *in vitro*	+	Enninga *et al.* (1986)

Table 33 (contd)

Test system	Result[a]	Reference
DIH, Pyrimidine dimer formation, human skin fibroblasts *in vitro*	+	Rosenstein & Mitchell (1987)
GIH, Gene mutation, human lymphoblastoid cell line *in vitro*	–	Tyrrell (1984)
GIH, Gene mutation, human skin fibroblasts *in vitro*	+	Enninga *et al.* (1986)
GIH, Gene mutation, human epithelial cells *in vitro*	+[b]	Jones *et al.* (1987)
DVH, Pyrimidine dimer formation, human skin *in vivo*	+	Freeman *et al.* (1989)

[a] +, positive; (+), weakly positive; –, negative
[b] Positive result with 365 nm but not with 334 nm at same fluence

Table 34. Genetic and related effects of predominantly UVB irradiation

Test system	Result[a]	Reference
SA9, *Salmonella typhimurium* TA98, reverse mutation	+	Calkins *et al.* (1987)
EC2, *Escherichia coli* WP2, reverse mutation	+	Peak *et al.* (1984)
TSC, *Tradescantia*, chromosomal aberrations	+	Kirby-Smith & Craig (1957)
DIA, DNA damage, Chinese hamster ovary cells *in vitro*	+	Zelle *et al.* (1980)
DIA, DNA strand breaks, Chinese hamster V79 cells	+	Matsumoto *et al.* (1991)
DIA, DNA-protein cross-links, Chinese hamster V79 cells	+	Matsumoto *et al.* (1991)
GCO, Gene mutation, Chinese hamster ovary cells *in vitro*	+	Zelle *et al.* (1980)
GCO, Gene mutation, Chinese hamster ovary cells *in vitro*	+	Rasmussen *et al.* (1989)
G9H, Gene mutation, Chinese hamster V79 lung cells, *hprt* locus	+	Wells & Han (1984)
G9H, Gene mutation, Chinese hamster V79 lung cells, *hprt* locus	+	Zölzer & Kiefer (1984)
G9O, Gene mutation, Chinese hamster V79 lung cells, ouabainr	+	Wells & Han (1984)
G9H, Gene mutation, Chinese hamster V79 lung cells *in vitro*, $6TG^r$	+	Colella *et al.* (1986)
G51, Gene mutation, mouse lymphoma L5178Y cells *in vitro*	+	Jacobson *et al.* (1981)
SIC, Sister chromatid exchange, Chinese hamster ovary cells *in vitro*	+	Rasmussen *et al.* (1989)
CIA, Chromosomal aberrations, ICR 2A frog cells *in vitro*	+	Rosenstein & Rosenstein (1985)
TCS, Cell transformation, Syrian hamster embryo cells *in vitro*	+	Doniger *et al.* (1981)
DIH, DNA strand breaks, human skin fibroblasts *in vitro*	+	Rosenstein & Ducore (1983)
DIH, Pyrimidine dimer formation, human skin fibroblasts *in vitro*	+	Enninga *et al.* (1986)
DIH, Pyrimidine dimer formation, human skin fibroblasts *in vitro*	+	Rosenstein & Mitchell (1987)
DIH, DNA strand breaks, human teratoma *in vitro*	+	Peak *et al.* (1987)
DIH, DNA double strand breaks, human teratocarcinoma *in vitro*	+	Peak & Peak (1990)
DIH, DNA-protein cross-links, human teratocarcinoma *in vitro*	+	Peak & Peak (1991)
DIH, Pyrimidine dimer formation in human skin keratinocytes *in vitro*	+	Schothorst *et al.* (1991)
DIH, Thymine dimer formation, human fibroblasts *in vitro*	+	Roza *et al.* (1988)
GIH, Gene mutation, human lymphoblastoid cell line *in vitro*	-	Tyrrell (1984)
GIH, Gene mutation, human skin fibroblasts *in vitro*	+	Enninga *et al.* (1986)
GIH, Gene mutation, human epithelial cells *in vitro*	+	Jones, C.A. *et al.* (1987)
TIH, Cell transformation, human fibroblasts *in vitro*	+	Sutherland *et al.* (1981)
DVA, Cyclobutane dimers in SV40 plasmid DNA in human fibroblasts *in vivo*	+	Mitchell *et al.* (1991)
DVA, Cytosine photohydrates in SV40 plasmid DNA in human fibroblasts *in vivo*	+	Mitchell *et al.* (1991)

Table 34 (contd)

Test system	Result[a]	Reference
DVA, Pyrimidine dimer induction, mouse skin *in vivo*	+	Cooke & Johnson (1978)
DVA, Pyrimidine dimer formation, mouse skin *in vivo*	+	Ley *et al.* (1983)
DVA, (6–4) Photoproduct formation, mouse epidermis *in vivo*	+	Olsen *et al.* (1989)
DVH, Pyridime dimer formation, human skin *in vivo*	+	Freeman *et al.* (1989)
UVH, Unscheduled DNA synthesis, human cornea *in vivo*[b]	+	Grabner & Brenner (1981)

[a] +, positive; –, negative
[b] From people who had been dead for 15 min

Table 35. Genetic and related effects of UVC irradiation

Test system	Result[a]	Reference
ECB, *Escherichia coli*, thymine dimer formation	+	Setlow *et al.* (1963)
ECB, *Escherichia coli*, photoproduct formation	+	Setlow (1968)
ECB, *Escherichia coli*, thymine photoadduct formation	+	Smith (1964)
ECB, *Escherichia coli*, pyrimidine dimers	+	Brash & Haseltine (1982)
ECB, *Escherichia coli*, (6-4) photoproducts	+	Brash & Haseltine (1982)
ECF, *Escherichia coli*, miscellaneous strains, forward mutation	+	Miller (1985)
ECR, *Escherichia coli*, mutation	+	Witkin (1976)
ECR, *Escherichia coli*, mutation	+	Walker (1984)
ECR, *Escherichia coli*, mutation	+	Franklin & Haseltine (1986)
ECR, *Escherichia coli*, mutation	+	Bridges *et al.* (1987)
ECR, *Escherichia coli*, mutation	+	Schaaper *et al.* (1987)
SSB, *Saccharomyces cerevisiae*, pyrimidine dimer formation	+	Wheatcroft *et al.* (1975)
SSB, *Saccharomyces cerevisiae*, pyrimidine dimer formation	+	Resnick *et al.* (1987)
SCN, *Saccharomyces cerevisiae*, aneuploidy	+	Parry *et al.* (1979)
SCF, *Saccharomyces cerevisiae*, forward mutation	+	Lee *et al.* (1988)
SCR, *Saccharomyces cerevisiae*, reverse mutation	+	Siede & Eckardt (1986)
PLU, Plants, DNA damage	+	McLennan (1987)
PLU, *Nicotiana tabacum*, unscheduled DNA synthesis	+	Cieminis *et al.* (1987)
PLU, *Chlamydomonas reinhardtii*, pyrimidine dimer formation	+	Vlček *et al.* (1987)
PLM, *Chlamydomonas reinhardtii*, mutation	+	Vlček *et al.* (1987)
TSC, *Tradescantia*, chromosomal aberrations	+	Kirby-Smith & Craig (1957)
DM?, *Drosophila melanogaster* embryo cells *in vitro*, DNA damage	+	Koval (1987)
DIA, DNA damage, ICR 2A frog cells *in vitro*	+	Chao & Rosenstein (1986)
DIA, DNA strand breaks, Chinese hamster V79 cells	+	Elkind & Han (1978)
DIA, DNA damage, Chinese hamster ovary cells *in vitro*	+	Zelle *et al.* (1980)
GCO, Gene mutation, Chinese hamster ovary cells *in vitro*	+	Zelle *et al.* (1980)
GCO, Gene mutation, Chinese hamster ovary cells *in vitro*	+	Rasmussen *et al.* (1989)
GCO, Gene mutation, Chinese hamster ovary cells *in vitro*	+	Drobetsky & Glickman (1990)
G9H, Gene mutation, Chinese hamster V79 lung cells *in vitro*	+	Colella *et al.* (1986)
G9H, Gene mutation, Chinese hamster V79 lung cells, *hprt* locus	+	Suzuki *et al.* (1981)
G9H, Gene mutation, Chinese hamster V79 lung cells, *hprt* locus	+	Zölzer & Kiefer (1984)
G9O, Gene mutation, Chinese hamster V79 lung cells, ouabain^r	+	Suzuki *et al.* (1981)

Table 35 (contd)

Test system	Result[a]	Reference
G51, Gene mutation, mouse lymphoma L5178Y cells *in vitro*	+	Jacobson *et al.* (1981)
SIC, Sister chromatid exchange, Chinese hamster V79 cells *in vitro*	+	Nishi *et al.* (1984)
SIC, Sister chromatid exchange, Chinese hamster ovary cells *in vitro*	+	Rasmussen *et al.* (1989)
SIA, Sister chromatid exchange, ICR 2A frog cells *in vitro*	+	Chao & Rosenstein (1985)
SIA, Sister chromatid exchange, chick embryo fibroblasts *in vitro*	+	Natarajan *et al.* (1980)
CIC, Chromosomal aberrations, Chinese hamster fibroblasts *in vitro*	+	Chu (1965a)
CIC, Chromosomal aberrations, Chinese hamster fibroblasts *in vitro*	+	Chu (1965b)
CIC, Chromosomal aberrations, Chinese hamster V79 cells *in vitro*	+	Bender *et al.* (1973)
CIC, Chromosomal aberrations, Chinese hamster V79 cells *in vitro*	+	Griggs & Bender (1973)
CIC, Chromosomal aberrations, Chinese hamster ovary cells *in vitro*	+	Ikushima & Wolff (1974)
CIC, Chromosomal aberrations, Chinese hamster CHEF-125 cells *in vitro*	+	Trosko & Brewen (1967)
CIA, Chromosomal aberrations, chick embryo fibroblasts *in vitro*	+	Natarajan *et al.* (1980)
CIA, Chromosomal aberrations, A8W243 *Xenopus* cells *in vitro*	+	Griggs & Bender (1973)
CIA, Chromosomal aberrations, ICR 2A frog cells *in vitro*	+	Rosenstein & Rosenstein (1985)
CIA, Chromosomal aberrations, New Zealand black mouse fetal fibroblasts	+	Reddy *et al.* (1978)
TBM, Cell transformation, BALB/c 3T3 mouse cells	+	Withrow *et al.* (1980)
TCM, Cell transformation, C3H 10T½ mouse cells	+	Chan & Little (1976)
TCM, Cell transformation, C3H 10T½ mouse cells	+	Mondal & Heidelberger (1976)
TCM, Cell transformation, C3H 10T½ mouse cells	+	Chan & Little (1979)
TCM, Cell transformation, C3H 10T½ mouse cells	+	Suzuki *et al.* (1981)
TCM, Cell transformation, C3H 10T½ mouse cells	+	Borek *et al.* (1989)
TCS, Cell transformation, Syrian hamster embryo cells	+	DiPaolo & Donovan (1976)
TCS, Cell transformation, Syrian hamster embryo cells	+	Doniger *et al.* (1981)
TCS, Cell transformation, Syrian hamster embryo cells	+	Borek *et al.* (1989)
TEV, Cell transformation, SV-40/BALB/c 3T3 mouse cells	+	Withrow *et al.* (1980)
DIH, DNA strand breaks, human skin fibroblasts *in vitro*	+	Rosenstein & Ducore (1983)
DIH, DNA damage, human skin fibroblasts *in vitro*	+	Rosenstein *et al.* (1985)
DIH, Pyrimidine dimer formation, human skin fibroblasts *in vitro*	+	Enninga *et al.* (1986)
DIH, Pyrimidine dimer formation, human skin fibroblasts *in vitro*	+	Rosenstein & Mitchell (1987)
DIH, DNA strand breaks, human teratoma cells *in vitro*	+	Peak *et al.* (1987)
DIH, Thymine dimer formation, human skin fibroblasts *in vitro*	+	Roza *et al.* (1988)
DIH, DNA damage, human skin fibroblasts *in vitro*	+	Chao & Rosenstein (1986)

Table 35 (contd)

Test system	Result[a]	Reference
DIH, DNA strand breaks, human fibroblasts *in vitro*	+	Lai & Rosenstein (1990)
DIH, DNA–protein cross-links, human fibroblasts *in vitro*	+	Lai & Rosenstein (1990)
DIH, DNA double strand breaks, human teratocarcinoma cells *in vitro*	+	Peak & Peak (1990)
DIH, DNA–protein cross-links, human teratocarcinoma cells *in vitro*	+	Peak & Peak (1991)
DIH, Pyrimidine dimer formation, human skin keratinocytes and melanocytes *in vitro*	+	Schothorst *et al.* (1991)
GIH, Gene mutation, human fibroblasts *in vitro*	+	Maher *et al.* (1979)
GIH, Gene mutation, human fibroblasts *in vitro*	+	Myhr *et al.* (1979)
GIH, Gene mutation, human lymphocytes *in vitro*	+	Sanderson *et al.* (1984)
GIH, Gene mutation, human lymphoblastoid cell line *in vitro*	+	Tyrrell (1984)
GIH, Gene mutation, human skin fibroblasts *in vitro*	+	Enninga *et al.* (1986)
GIH, Gene mutation, human epithelial cells *in vitro*	+	Jones, C.A. *et al.* (1987)
GIH, Gene mutation, human HeLa cells *in vitro*	+	Musk *et al.* (1989)
GIH, Gene mutation, human lymphocytes *in vitro*	+	Norimura *et al.* (1990)
GIH, Gene mutation, human fibroblasts *in vitro*	+	Dorado *et al.* (1991)
GIH, Gene mutation, human fibroblasts *in vitro*	+	McGregor *et al.* (1991)
GIH, Gene mutation, human melanoma cells *in vitro*	+	Musk *et al.* (1989)
SHF, Sister chromatid exchange, human fibroblasts *in vitro*	+	Fujiwara *et al.* (1981)
SHF, Sister chromatid exchange, human fibroblasts *in vitro*	+	Kurihara *et al.* (1987)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+	Murthy *et al.* (1982)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+	Perticone *et al.* (1986)
SHF, Sister chromatid exchange, human skin fibroblasts	+	De Weerd-Kastelein *et al.* (1977)
SHF, Sister chromatid exchange, human skin fibroblasts	+	Krepinsky *et al.* (1980)
SHF, Sister chromatid exchange, human skin fibroblasts	+	Marshall *et al.* (1980)
SHF, Sister chromatid exchange, human skin fibroblasts	+	Henderson *et al.* (1985)
MIH, Micronucleus test, human skin fibroblasts *in vitro*	+	Krepinsky *et al.* (1980)
CHF, Chromosomal aberrations, human fibroblasts *in vitro*	+	Parrington (1972)
CHF, Chromosomal aberrations, human skin fibroblasts	+	Parrington *et al.* (1971)
CHF, Chromosomal aberrations, human skin fibroblasts	+	Marshall & Scott (1976)
CHL, Chromosomal aberrations, human lymphocytes *in vitro*	+	Murthy *et al.* (1982)
CHL, Chromosome exchanges, human lymphocytes *in vitro*	+	Holmberg & Gumauskas (1990)
TIH, Cell transformation, human fibroblasts *in vitro*	+	Sutherland *et al.* (1981)
TIH, Cell transformation, human fibroblasts *in vitro*	+	Maher *et al.* (1982)

Table 35 (contd)

Test system	Result[a]	Reference
TIH, Cell transformation, human fibroblasts *in vitro*	+	Sutherland *et al.* (1988)
???, Cyclobutane dimers in SV40 plasmid DNA in human skin fibroblasts *in vitro* and *in vivo*	+	Mitchell *et al.* (1991)
???, Cytosine photohydrates in SV40 plasmid DNA in human skin fibroblasts *in vitro* and *in vivo*	+	Mitchell *et al.* (1991)
DVA, Pyrimidine dimer formation, mouse skin *in vivo*	+	Bowden *et al.* (1975)

[a] +, positive