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Wavelength dependence of pyrimidine dimer formation in DNA of human skin irradiated *in situ* with ultraviolet light

(solar effects/gel electrophoresis/ozone depletion/skin cancer)

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ABSTRACT **The UV components of sunlight are believed to be a major cause of human skin cancer, and DNA is thought to be the principal molecular target. Alterations of the intensity and wavelength distribution of solar UV radiation reaching the surface of the earth, for example by depletion of stratospheric ozone, will change the effectiveness of solar radiation in damaging DNA in human skin. Evaluation of the magnitude of such effects requires knowledge of the altered sunlight spectrum and of the action spectrum for damaging DNA in human skin. We have determined an action spectrum for the frequency of pyrimidine dimer formation induced in the DNA of human skin per unit dose of UV incident on the skin surface. The peak of this action spectrum is near 300 nm and decreases rapidly at both longer and shorter wavelengths. The decrease in our action spectrum for wavelengths <300 nm is attributed to the absorption of the upper layers of the skin, an *in situ* effect that is inherently included in our measurements. Convolution of the dimer action spectrum with the solar spectra corresponding to a solar angle of 40° under current levels of stratospheric ozone (0.32-cm O_3 layer) and those for 50% ozone depletion (0.16-cm O_3 layer), indicate about a 2.5-fold increase in dimer formation. If the action spectrum for DNA damage that results in skin cancer resembles that for dimer induction in skin, our results, combined with epidemiological data, suggest that a 50% decrease in stratospheric ozone would increase the incidence of nonmelanoma skin cancers among white males in Seattle, Washington, by 7.5- to 8-fold, to a higher incidence than is presently seen in the corresponding population of Albuquerque, New Mexico.**

Sunlight causes erythema, premature aging, and cancers in human skin (1), and several lines of evidence indicate that UV radiation of wavelengths <320 nm is of primary importance in damage induction (2). Studies of the wavelength dependence of carcinogenesis in mouse skin (3) and of erythema induction in human skin (4) indicate that the efficiency of damage induction by photons in the wavelength range 290–400 nm varies strikingly. Thus, alteration of the solar spectrum reaching the earth's surface—for example, through alteration in the ozone content of the stratosphere—will affect the frequency of DNA damages, including those in human skin.

Quantitative prediction of the extent of damage and of its biological consequences requires convolution of the altered solar spectrum with the action spectrum for inducing human skin cancer (2). Because this action spectrum is not known, other biological response functions have been used to approximate the human skin cancer spectrum: action spectra (*i*) for carcinogenesis in mouse skin (3), (*ii*) for killing and mutation in prokaryotes (2), and (*iii*) for killing, mutation, and transformation of mammalian cells in culture (5, 6), as well as several different action spectra (*iv*) for erythema in human skin (4, 7). However, the optical properties of mouse skin and of prokaryotes and mammalian cells in culture differ from those of human skin *in situ*, and the cellular target or targets for erythema induction in humans remain unknown.

DNA damage can lead to oncogenic alterations that play important roles in induction of human cancers. To evaluate the role of sunlight—and of changes in the spectrum of sunlight—it is necessary to measure the action spectrum for inducing damage in DNA in human skin *in situ*. One significant UV-induced DNA lesion is the cyclobutyl pyrimidine dimer, formed between adjacent pyrimidines on the same DNA strand. We have developed methods for quantitating these lesions at low frequencies in nanogram amounts of nonradioactive DNA (8, 9). We report dimer yields as a function of wavelength in the skin of 30 humans, determined by using these methods. These data allow calculation of the increase in DNA damage resulting from alterations in the solar spectrum through, for example, ozone depletion and allow estimation of the resulting increase in human skin cancers.

MATERIALS AND METHODS

Irradiation of Human Skin. Untanned gluteal skin of healthy adult Caucasian volunteers from whom informed consent had been obtained was exposed to narrow-band UV radiation from a monochromator [5000 W Xe-Hg compact arc in an Optical Radiation V-4500 housing, optically matched to an f/2.0 Jobin Yvon HL300 holographic grating monochromator; radiation was directed to the monochromator entrance slit by spectrally selective dielectric mirrors (Optical Radiation model 903934-001 for wavelengths 275–334 nm and model 903934-002 for 365–405 nm)] or from an excimer-pumped dye laser [Lambda Physik model EMG 103 excimer laser operated at 308 nm with XeCl provided pulses of 20-ns duration at a repetition rate of 20–30 pulses per sec to pump a Lambda Physik model FL 2002 dye laser (which, to obtain 385 nm, contained 4,4′-bis-[2-butyloctyloxy]-*p*-quaterphenyl in dioxane; and to obtain 405 nm, contained 4,4′-diphenylstilbene in dioxane)]. Half-power bandwidths for the monochromator were as follows: 275–313 nm, 6 nm; 334 nm, 10 nm; 366–405 nm, 20 nm. Broader bandwidths were required at the longer wavelengths to compensate, in part, for the greatly reduced cross section for dimer production. Radiation from the monochromator was measured with an Eppley

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Abbreviations: MED, minimal erythema dose; ESS, *Micrococcus luteus* UV endonuclease-sensitive sites.

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model E4 thermopile coupled to a Keithley 150B microvoltmeter. Radiation from the dye laser was measured by using a Coherent model 210 power meter. Uniformity of the exposure field for both light sources was within ±10%.

The exposures for each subject were chosen on the basis of the subject's minimal erythema dose (MED) at each wavelength. MEDs were determined by exposing nine 1-cm diameter sites to a graduated series of UV doses, increasing geometrically by 25%. The MED was identified as the lowest exposure that induced uniform pinkness filling the exposure site with well-defined margins 24 hr after irradiation.

Untanned skin sites (2 × 2 cm) were exposed to 1 or 2 MED of UV radiation, 0.1 ml of 1% lidocaine was injected intradermally, and superficial shave biopsies (6–8 mm in diameter) were excised from the exposed sites and from an unirradiated control site using dim red light (to prevent photorepair) for illumination. Times for the lower of the two exposures, with the range and average duration (in min), were as follows: 275 nm, range of 2.2–10.3, and average of 6.7; 282 nm, range of 1–5.7 and average of 2.7; 290 nm, range of 0.55–2.88 and average of 1.7; 296 nm, range of 0.13–2.18 and average of 1.24; 304 nm, 0.1–3.6 and average of 1.42; 313 nm, range of 3.43–25.8 and average of 11.15; 334 nm, range of 26.53–73.8 and average of 44.82; 365 nm, range of 17.9–82.8 and average of 58.53; the higher exposure was usually twice that of the lower exposure. Each biopsy required ≈2 min.

DNA Preparation and Pyrimidine Dimer Measurements. Individual biopsies were immediately immersed in 1 ml of cold 0.25% trypsin (Whitaker M.A. Bioproducts) in 0.17 M NaCl/3.4 mM KCl/10.1 mM Na_2HPO_4/1.8 mM KH_2PO_4, pH 7.3 and incubated on ice in the dark for 12–24 hr. The epidermis was separated from the underlying dermis by gentle scraping and homogenized in a glass/glass micro tissue grinder; the DNA was extracted from the epidermis as described by Gange *et al.* (10).

The frequencies of *Micrococcus luteus* UV endonuclease-sensitive sites (ESS) per kilobase (kb) of DNA were determined as described (8, 9). In brief, DNA was extracted, treated with *Micrococcus luteus* UV endonuclease (11) at sufficient concentrations to cleave quantitatively at pyrimidine dimer sites (12), while companion samples were incubated without endonuclease. The DNA was denatured by treatment with alkali and then subjected to electrophoresis along with molecular-length standards [T4, 170 kb; λ, 48.5 kb; T7, 40 kb; *Bgl* I fragments of T7 (22.5, 13.5, and 4 kb)] for 2 hr at 1–3 V/cm [static field (8)] or 16 hr with 0.3-s pulses (9 V/cm) and 10-s interpulse period [unidirectional pulsed field (9)] in a 10 × 14 cm 0.4% alkaline agarose gel in 2 mM EDTA/30 mM NaOH as electrophoresis buffer. The gel was neutralized (0.1 M Tris, pH 8, for 30 min), stained with ethidium bromide (1 μg/ml in water), destained and photographed using Polaroid type 55 positive/negative film, or a digital image of ethidium fluorescence was obtained using an electronic imaging system (13). The number-average molecular lengths, in kb, for the UV endonuclease-treated and untreated samples were determined from analysis of the DNA profiles as described in ref. 8, and the frequency of dimers, N (in units of ESS/kb), was calculated by using the equation:

$$N = \frac{1}{L_n(+\text{endo})} - \frac{1}{L_n(-\text{endo})}, \qquad [1]$$

where $L_n(+\text{endo})$ is the number-average molecular length (in kb) of the DNA sample treated with UV endonuclease, and $L_n(-\text{endo})$ is the number-average molecular length (in kb) of the corresponding untreated sample (8). Data are given as the average of measurements obtained from three independent determinations (gels) for each sample.

RESULTS

Determination of an action spectrum for pyrimidine dimer formation in DNA in the skin of humans requires measurement of dose–response curves for damage production in many volunteers over a wide wavelength range. To ensure that all exposures were in a biologically relevant range, we determined each individual's MED for a particular wavelength and exposed that person to doses up to 2× MED at that wavelength. We obtained epidermal biopsies, extracted DNA, treated the DNA with UV endonuclease, and determined the pyrimidine dimer yields from triplicate independent gels; the dimer induction efficiencies (slopes of the lines relating dimer yield to UV exposure) for 275, 280, 289, 296, 302, 313, 334, and 366 nm determined in 30 volunteers (at least 5 volunteers per wavelength; data at 302 nm were obtained for all but one volunteer) are shown in Fig. 1.

The action spectrum for the induction of dimers by light incident on human skin, plotted in Fig. 1, varies over five orders of magnitude in the wavelength range 275–366 nm, with the maximum of this absolute action spectrum between 296 and 302 nm. The inter-individual values at each wavelength spread over a significant range, probably reflecting the correlation of photoproduct yield and factors such as pigmentation and sun sensitivity of the volunteer, as previously observed for broad-spectrum UV radiation (15). Several groups have reported different rates for excision and photoreactivation of dimers in the DNA of human skin irradiated *in situ* (16–19): excision of one half of the dimers initially

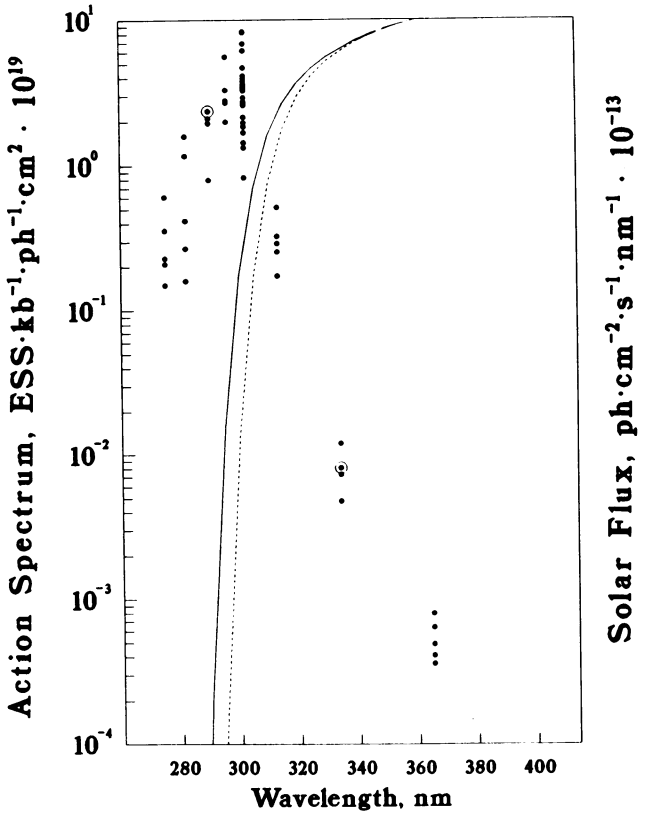

FIG. 1. Action spectrum for pyrimidine dimer formation in human skin (●) and solar spectra at the surface of the earth for stratospheric ozone levels of 0.32 cm (---) and 0.16 cm (—) (14). Each point in the action spectrum represents the slope of the dose–response line (dimer yields at three exposures) for one volunteer at one wavelength, obtained from triplicate independent determinations. A total of 30 points occurs at 302 nm, although some points overlie other values. There are five points 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.

present in times as short as 1 hr (17) and as long as ≈12 hr (18) have been reported. Without better knowledge of the actual rate, it is not yet possible to correct our values for repair occurring during irradiation. At wavelengths near 300 nm, however, the irradiation times are brief compared with estimates of times required for excision, and photoreactivation is unlikely to be a major effect. The dimer yields at the longer wavelengths are so low that, even if excision reduced actual values by a factor of two (corresponding to the higher measurement of excision rates), the efficiency of dimer production at these wavelengths would be small compared with the yields around 300 nm.

We also examined the ability of long-wavelength UV (385 nm) and blue (405 nm) radiation to induce dimers in DNA in human skin, and we found that these wavelengths are largely ineffective in net production of pyrimidine dimers in DNA of human skin irradiated *in situ*. Such wavelengths, however, have been reported to induce cell killing, presumably by other mechanisms (6, 20).

Determination of the effect of changes in the solar spectrum on DNA damage in skin requires not only knowledge of the wavelength dependence of dimer production, but also of the intensity of solar radiation of different wavelengths reaching the earth's surface. Fig. 1 shows two spectral distributions for downward global flux of UV (photons per $cm^{-2}\cdot sec^{-1}\cdot nm^{-1}$) on the earth's surface at a solar angle of 40° for two stratospheric ozone levels: the current effective ozone depth of 0.32 cm and the corresponding function at the same solar angle for an ozone depth of 0.16 cm (14), levels recently observed for the Antarctic ozone hole (21). The product of the action spectrum and a solar flux spectrum at each wavelength is the number of dimers formed per kb of DNA per sec of exposure for each nm-wide segment of the spectrum; this is the effectivity spectrum ($ESS\cdot kb^{-1}\cdot sec^{-1}\cdot nm^{-1}$) for formation of dimers by a particular spectrum of incident light. The total frequency of dimers induced by an exposure is obtained by integrating the effectivity spectrum over both wavelength and the duration of the exposure.

Fig. 2 shows the effectivity spectra obtained by convoluting the action spectrum for dimer induction by light incident on the skin with the solar spectra for ozone thicknesses of 0.32 cm and 0.16 cm and a solar angle of 40°. We chose these values to illustrate the significant changes in the wavelength composition of sunlight that result from changes in the ozone column. Assuming additivity of the photochemical events—that the total photoproduct yield depends only on the quantity of light of each wavelength present and the efficiency of each wavelength in inducing the photoproduct—the relative areas of the effectivity spectra provide an estimate of initial dimer yields under the two conditions. The area for 0.16-cm ozone is ≈2.5 times that for 0.32-cm ozone.

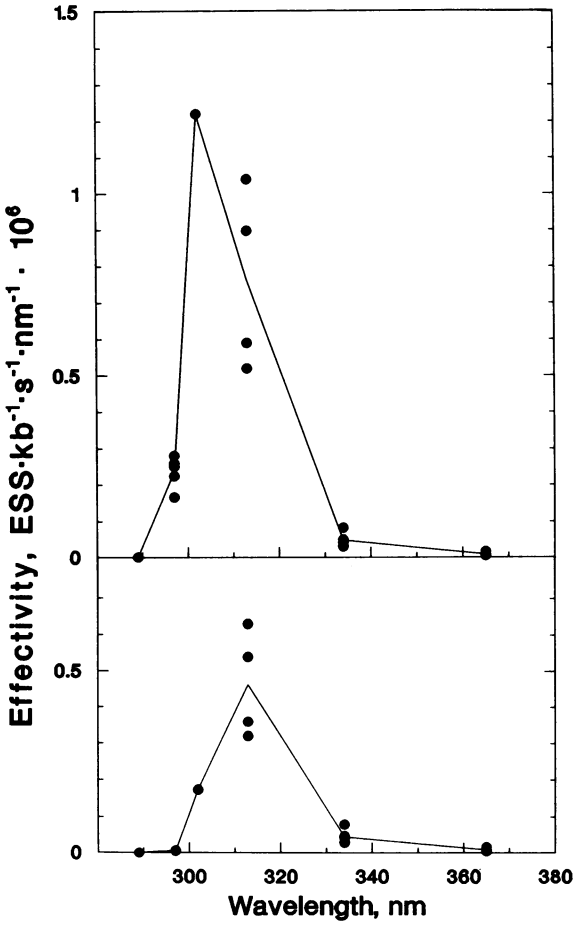

FIG. 2. Effectivity spectra for pyrimidine dimer formation in DNA in human skin for a solar angle of 40° and stratospheric levels of 0.32-cm (*Lower*) and 0.16-cm (*Upper*) ozone. Normalized pyrimidine dimer yields (the mean value of slope of the dose–response line for all volunteers at 302 nm times the slope of the dose–response line for a volunteer at a given wavelength divided by the slope for that volunteer at 302 nm) are multiplied by solar flux at that wavelength. Normalization reduces variability in the action spectrum due to differences in response to UV among volunteers; normalization causes the 302-nm values for all volunteers to map to a single point in each spectrum. The solid line in each spectrum connects the mean values for all volunteers at each wavelength. The ratio of the areas defined by the line for 0.16-cm ozone is ≈2.5 times that for 0.32-cm ozone.

DISCUSSION

Several lines of evidence indicate that sunlight induces cancers in human skin. For squamous and basal cell carcinoma, the correlation of rates of incidence with latitude and presumably solar exposure (22), occurrence on sun-exposed body regions (22), and higher incidence in individuals who spend more time outdoors (23) point to sun as a major etiologic agent. For melanoma, there is also good correlation of rates of mortality with latitude (24). Increased exposure of human populations to the UV components of sunlight is thus likely to produce higher rates of incidence of such cancers. Prediction of the magnitude of such rate increases from changes in the solar spectrum requires (*i*) knowledge of the spectrum of radiation reaching the earth's surface and (*ii*) determination of the effectiveness in cancer induction of the different wavelengths in that spectrum.

The dose–response functions for induction of human skin cancers by different UV wavelengths have not been determined. An action spectrum for skin carcinogenesis in the mouse (3) provides a similar biological endpoint, but the structure—and thus the transmission—of mouse skin is rather different from human skin. Action spectra for erythema in human skin provide a measure of damage response in humans, but the relation of damages leading to erythema to those inducing skin cancer is unknown; further, different investigators have obtained spectra with striking differences in the 250–320 nm region (see refs. 4 and 7), making calculations based on such spectra subject to large uncertainties. These differences are especially large in the wavelength range of 280–320 nm, the wavelength region most affected by ozone depletion.

If the quantum yield for DNA damage were constant across the DNA absorption band and if no other absorbing moieties contributed to DNA damage, DNA absorption spectra would be useful predictors of biological response. However, action spectra for dimer formation in cells and killing (25), mutation, and transformation (5) of human and other eukaryotic cells

show small but significant differences from the absorption spectrum of mammalian DNA (26).

For human skin two additional important factors must also be taken into account: (*i*) The overlying stratum corneum and upper epidermal cell layers absorb incident UV radiation of different wavelengths selectively and thus will alter the spectral distribution of radiation reaching lower cellular layers. (*ii*) Nuclei of cells in skin might contain sensitizer molecules capable of transferring excitation energy to DNA indirectly, thus allowing DNA damage by radiation of wavelengths that DNA does not absorb. Thus the action spectrum for DNA damage in human skin *in situ* is the most direct measurement currently available of the efficiency of different wavelengths in sunlight for producing potentially oncogenic damage.

One biologically significant DNA lesion is the cyclobutyl pyrimidine dimer, which can be quantitated at low frequencies in nanogram quantities of nonradioactive DNA by cleavage with a dimer-specific UV endonuclease followed by alkaline agarose gel electrophoresis (8, 9). We have used this method to determine pyrimidine dimer yields induced by 10 wavelengths over the range 275–405 nm in the epidermis of Caucasian skin irradiated *in situ*. Fig. 1 contains a summary of the data for 275–365 nm. These results illustrate that there are significant differences among individuals of similar skin type; the data provide an envelope of the response range for the volunteers tested, all of whom were classified as skin types II and III (see, for example, ref. 4).

Fig. 1 also shows curves for solar spectra at the earth's surface for a solar angle of 40° under present ozone conditions (an effective layer thickness of 0.32 cm) and in conditions of an ozone hole with an effective layer of 0.16 cm. Because ozone absorbs strongly below ≈300 nm but absorbs rather little above ≈340 nm, the major effect of significant depletion of stratospheric ozone would be a marked increase in radiation to the earth's surface in the 280- to 300-nm region and virtually no change in radiation levels above ≈340 nm. The convolution of the solar spectra for the current stratospheric levels of ozone and for altered ozone concentrations with the action spectra for dimer production in skin is shown in Fig. 2. Decreases in ozone levels shift the peak efficiency of dimer production to shorter wavelengths; dimer yields under the ozone-hole conditions of 0.16-cm thickness would be ≈2.5 times the yields from the present ozone levels of 0.32 cm. The total increase in UV at any one location would, of course, have to be determined by integration over a wide range of solar angles because this parameter changes constantly during the day as well as with the seasons. Local conditions such as cloud cover and pollution of the lower atmosphere will also modify the total integrated UV dose.

Tyrrell and Pidoux (6) determined effectivity spectra for sunlight-induced cytotoxicity for human cells in culture by convoluting an action spectrum for cytotoxicity with the transmission spectrum through 70 μm of human skin and various experimentally determined solar spectra. For the highest solar angles from their data, noon in Albuquerque, New Mexico, in mid May, their effectivity spectrum also has a maximal value just above 300 nm, but this maximum decreases less rapidly at longer wavelengths than in the spectrum of Fig. 2, dropping by only an order of magnitude by 434 nm. Thus, the longer wavelengths are responsible for a much larger fraction of the cytotoxic effects of sunlight than for the production of pyrimidine dimers, particularly for exposures that occur before 10 a.m. or after 2 p.m. It follows that the fractional increase in cytotoxicity resulting from ozone depletion will be smaller than that for the formation of dimers. Because wavelengths >330 nm do not contribute significantly to the induction of skin tumors in hairless mice (3), our effectivity spectrum for dimer production in human skin appears more applicable to predictions of photocarcinogenesis in humans than is the cytotoxicity spectrum.

Can action spectra for DNA damage be combined with predictions of solar spectra to predict changes in cancer rates resulting from changes in the ozone column? Obviously, laboratory experiments to determine the dose–response function for cancer induction by different wavelengths in humans are not practical. An approach to this problem can be based on the hypothesis introduced by Setlow (2) that the induction of skin cancer will be some (probably nonlinear) function of the total frequency of damage inflicted upon DNA by all wavelengths. The frequency of dimers induced in DNA by all the wavelengths present during an irradiation is the effectivity spectrum integrated over both the duration of an exposure and the spectrum of wavelengths present. Empirical dose–response functions for induction of particular types of skin cancers as a function of UV burden in a target population are known approximately from correlations between epidemiological data and measurements of solar flux at different geographical locations within the United States (7, 22). Among the locations for which both solar UV data and the age-adjusted incidence of nonmelanoma skin cancer in the white male population were available, Albuquerque had the highest and Seattle had the lowest annual solar UV index. The solar UV index for Albuquerque is double the value for Seattle, but the incidence of nonmelanoma skin cancer in the Albuquerque white male population is three times as great.

At the time the solar UV flux data were recorded, recording the entire solar spectrum at each location with high resolution was impractical. Thus, estimates of the total incident UV exposure used in the epidemiological studies were based either on the radiation flux in a narrow band centered near 305 nm or on readings from Robertson–Berger meters (27), which record a weighted average of the UV solar spectrum. The weighting function of the Robertson–Berger meter was selected to approximate an action spectrum for erythema, but is, in fact, significantly shifted to longer wavelengths compared with erythema action spectra (27) and also compared with the dimer action spectrum we determined. The 305-nm intensity data and the Robertson–Berger meter data are, fortunately, valuable tools for comparing epidemiological data from various geographical locations because all UV wavelengths tend to change in approximately the same relative proportion as a function of solar angle for a constant ozone column (14). However, 305-nm intensities and Robertson–Berger meter readings cannot be used to estimate the effects of changing stratospheric ozone levels because reductions in the ozone layer will alter the shape of the solar spectrum. Estimates of the relative damage to DNA induced by solar spectra associated with different levels of stratospheric ozone can be obtained, however, assuming that the action spectrum for formation of pyrimidine dimers is also typical of the spectrum for other relevant types of UV-induced damage.

The ratio of the wavelength integrals of the effectivity spectra in Fig. 2 indicates that a decrease in stratospheric ozone of 50% will result in a level of DNA damage 2.5 times the current level for a solar angle of 40°, approximately the correct angle for Seattle, which is located near the 50th parallel. Scotto (22) fit the incidence of nonmelanoma cancers for 10 locations in the United States as an exponential function of UV index. These data predict that a 2.5-fold higher UV index and hence presumably level of DNA damage would result in an incidence among white males in Seattle of 7.5–8.0 times the current level. Extrapolations of cancer rates in a particular population at a given location based on data from populations at other locations are subject to a variety of uncertainties (28). Regardless of the details of the extrapolation used, however, it seems likely that a 50% decrease in stratospheric ozone over the northern United

States would result in incidences of nonmelanoma skin cancer among white males that would exceed the current levels among comparable populations in Albuquerque and other southwestern cities. While this analysis is based on an extreme case of ozone depletion, clearly lesser reductions in stratospheric ozone would also result in significant increases in skin cancer rates.

Pyrimidine dimers are the most numerous photoproducts formed in DNA by wavelengths <320 nm, but such dimers are not the only lesions formed by these wavelengths. The second most numerous class are pyrimidine(6–4)pyrimidones. Although the biological significance of pyrimidine(6–4)pyrimidone photoproducts *vis-a-vis* pyrimidine dimers is not yet clear, the action spectrum for the formation of pyrimidine(6–4)pyrimidone photoproducts is almost identical to that for dimers as judged both by direct chemical assay (29) and by RIA (30) when allowance is made for the subsequent photolysis of the pyrimidine(6–4)pyrimidone photoproducts (31). Thus, the calculations of the relative increases in DNA damage resulting from decreased stratospheric ozone apply to the biological consequences of both pyrimidine(6–4)pyrimidone photoproducts and pyrimidine dimers.

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1. Urbach, F. (1969) *The Biological Effects of Ultraviolet Radiation (with emphasis on the skin)* (Pergamon, New York).
2. Setlow, R. B. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 3363–3366.
3. Cole, C. A., Forbes, P. D. & Davies, R. E. (1986) *Photochem. Photobiol.* **43,** 275–284.
4. Gange, R. W., Park, Y.-K., Auletta, M., Kagetsu, N., Blackett, A. D. & Parrish, J. A. (1986) in *The Biological Effects of UVA Radiation*, eds. Urbach, F. & Gange, R. W. (Praeger, New York), pp. 57–65.
5. Sutherland, B. M., Delihas, N. C., Oliver, R. P. & Sutherland, J. C. (1981) *Cancer Res.* **41,** 2211–2214.
6. Tyrrell, R. M. & Pidoux, M. (1987) *Cancer Res.* **47,** 1825–1829.
7. National Academy of Sciences (1975) *Enviromental Impact of Stratospheric Flight* (National Academy of Sciences, Washington, DC).
8. Freeman, S. E., Blackett, A. D., Monteleone, D. C., Setlow, R. B., Sutherland, B. M. & Sutherland, J. C. (1986) *Anal. Biochem.* **158,** 119–129.
9. Sutherland, J. C., Monteleone, D. C., Mugavero, J. H. & Trunk, J. (1987) *Anal. Biochem.* **162,** 511–520.
10. Gange, R. W., Blackett, A. D., Matzinger, E. A., Sutherland, B. M. & Kochevar, I. E. (1985) *J. Invest. Dermatol.* **85,** 363–364.
11. Carrier, W. L. & Setlow, R. B. (1970) *J. Bacteriol.* **102,** 178–186.
12. Ahmed, F. E. & Setlow, R. B. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 1548–1552.
13. Sutherland, J. C., Lin, B., Monteleone, D. C., Mugavero, J., Sutherland, B. M. & Trunk, J. (1987) *Anal. Biochem.* **163,** 446–457.
14. Shettle, E. P., Nack, M. L. & Green, A. E. S. (1975) in *Impacts of Climatic Change on the Biosphere* (National Technical Information Service, Springfield, VA), Part 1, Rep. DOT TST-76-55, pp. 2-38–2-49.
15. Freeman, S. E., Gange, R. W., Matzinger, E. A. & Sutherland, B. M. (1986) *J. Invest. Dermatol.* **86,** 34–36.
16. Sutherland, B. M., Harber, L. C. & Kochevar, I. (1980) *Cancer Res.* **40,** 3181–3185.
17. D'Ambrosio, S. M., Slazinski, L., Whetstone, J. W. & Lowney, E. (1981) *J. Invest. Dermatol.* **77,** 311–313.
18. Freeman, S. E. (1981) *J. Invest. Dermatol.* **90,** 814–817.
19. D'Ambrosio, S. M., Whetstone, J. W., Slazinski, L. & Lowney, E. (1981) *Photochem. Photobiol.* **34,** 461–464.
20. Webb, R. B. (1977) in *Photochemical and Photobiological Reviews*, ed. Smith, K. C. (Plenum, New York), Vol. 2, pp. 169–261.
21. Farman, J. C., Gardiner, B. G. & Shanklin, J. D. (1985) *Nature* **315,** 207–210.
22. Scotto, J. (1986) in *Effects of Changes in Stratospheric Ozone and Global Climate*, ed. Titus, J. G. (U.S. Environmental Protection Agency, Washington, DC), Vol. 2, pp. 33–61.
23. Urbach, F., Rose, D. B. & Bonnem, M. (1972) in *Environment and Cancer: A Collection of Papers*, eds. Hewitt, R. R. & Romsdahl, M. (Williams and Wilkins, Baltimore), pp. 355–371.
24. Mason, T. J. & McKay, F. W. (1974) *U.S. Cancer Mortality by County: 1950–1969* (U.S. Government Printing Office, Washington, DC), DHEW Pub. No. NIH 74-615.
25. Kantor, G. J., Sutherland, J. C. & Setlow, R. B. (1980) *Photochem. Photobiol.* **31,** 459–464.
26. Sutherland, J. C. & Griffin, K. P. (1981) *Radiat. Res.* **86,** 399–409.
27. Berger, D. (1976) *Photochem. Photobiol.* **24,** 587–593.
28. Scott, E. L. & Straf, M. L. (1977) in *Origins of Human Cancer A: Incidence of Cancer in Humans*, eds. Hiatt, H. H., Watson, J. D. & Winsten, J. A. (Cold Spring Harbor Lab., Cold Spring Harbor, NY), pp. 529–546.
29. Chan, G. L., Peak, M. J., Peak, J. G. & Haseltine, W. A. (1986) *Int. J. Radiat. Res.* **50,** 641–648.
30. Rosenstein, B. S. & Mitchell, D. (1987) *Photochem. Photobiol.* **45,** 775–780.
31. Mitchell, D. L. & Rosenstein, B. S. (1987) *Photochem. Photobiol.* **45,** 781–786.