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UV Action Spectra For Mammalian Systems: Their Implications for the Predicted Effects of Ozone Depletion on Skin Cancer Incidence

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INTRODUCTION

The predicted environmental effects of UV-B depend on the action spectrum for the response studied. Since such spectra change rapidly -- usually decreasing with increasing wavelength -- and since the biological effects depend on the product of the action spectrum and the sun's spectrum at the surface of the earth which decreases with decreasing wavelength, a slight change in action spectrum will markedly influence the predicted effects of ozone depletion on biological systems. Thus, the key, but by no means only step in the prediction, is a knowledge of the action spectrum. Unfortunately, it is rare that we know or even hope to know the spectrum for biological systems of interest such as skin cancer induction, nor is it possible to do experiments with solar simulators on many systems. Hence, we must base our predictions on the photobiological properties of simple systems and the knowledge of their action spectra and general biological theories connecting simple cells with higher organisms[1].

DNA DAMAGE

Environmental agents affect cells by altering their membranes, proteins, RNA or DNA. DNA molecules are the largest in cells, and they are present in the fewest copies. Their integrity is essential for transcription and, hence, protein translation. Even in nondividing cells, changes in the structure of DNA can be cytotoxic[2]. Moreover, changes in DNA lead to mutations, and there is strong evidence that damages to DNA represent initiating events in carcinogenesis[1]. Thus, a reasonable first step in the evaluation of the biological effects of UV-B radiation and the effects of depletion of

stratospheric ozone is to assume that cellular DNA is the most important target. We make the further simplified assumption (probably not true) that the effects of a band of UV-B wavelengths is the sum of the effects of the individual wavelength components in that band. Thus, from this point of view, our goal is to determine the action spectrum for affecting DNA in cells of higher organisms.

This brief report is based on the author's experimental experience in the field of Photobiology, and as chairman of the U. S. National Research Council Committee on Biological Effects on Increased Solar Ultraviolet Radiation. The report of this committee has recently been published[1].

ACTION SPECTRA FOR DNA-DAMAGE IN VERTEBRATE CELLS

The available evidence indicates that the effects in the UV-B range are similar to those produced by 254 nm radiation. For example, human fibroblasts that are nonproliferating in culture because of deprivation of serum are affected by 254 nm radiation so they no longer adhere to the surface on which they were originally grown[2]. This cytotoxic effect is photoreversible in cells that contain photoreactivating enzyme (see below) indicating that pyrimidine dimers in DNA were responsible for the cytotoxicity[3]. Similar cytotoxic effects are observed for irradiation by 313 nm or by UV-B. Morevover, repair deficient human cells from individuals with the skin-cancer-prone-disease xeroderma pigmentosum (XP) are more sensitive than normal cells to individual wavelengths between 240 and 313 nm and the ratio of sensitivities of XP cells to normal cells is independent of wavelength[4]. A similar ratio is found for broad bands of UV-B[5], and in a survey of a number

of XP cell strains the ratios of sensitivities were the same at 254 nm and 313 nm[6]. Moreover, the cytotoxic sensitivity to 254 nm, FS-40 light and FS-40 light filtered to remove wavelengths below 290 nm is proportional to the numbers of pyrimidine dimer photoproducts in DNA as measured by the susceptibility of DNA to a particular UV endonuclease[5] (Fig. 1). However, this proportionality breaks down, for the long wavelength region of UV-B bands, for mutation in proliferating mouse or hamster cells[7] or for sister chromatid exchanges in proliferating hamster cells[8]. It is not clear whether the differences between the various systems are a function of proliferation or a function of cell type. Nevertheless, further evidence that there is nothing special about the UV-B region comes from a comparison of the photoreactivable sectors for frog cells irradiated by a range of UV wavelengths[9]. The photoreactivable sector is the fraction of damage that can be removed via enzymatic monomerization of pyrimidine dimers. The sector is, within experimental error, independent of inactivating wavelength from 252 nm to 313 nm, and its measured value indicates that 82% of the cytotoxic action of UV for such frog cells arises from pyrimidine dimers. It should be obvious that damages other than pyrimidine dimers can contribute to biological effects of DNA[10]. At present, the characteristics of such other lesions are not known, but it does seem as if these other damages may become more important at the longer wavelengths.

The photoreactivation experiments cited above were carried out on frog cells because in culture they contain large amounts of photoreactivating enzyme, whereas human cells usually do not. Both nonproliferating human and frog cells are subject to the cytotoxic effects of UV, and the cytotoxic

effects on frog cells are subject to photoreactivation[5]. These data strongly implicate damage to DNA and, in particular, pyrimidine dimers as the changes in DNA responsible for cytotoxic effects.

DNA ACTION SPECTRA

Earlier work on bacterial and viral systems by a number of investigators permitted Setlow to summarize such data so as to give an average DNA action spectrum[11]. Recent experimental work has concentrated on obtaining such spectra for vertebrate cells for a number of different endpoints. Action spectra have been obtained for both killing[4] and transformation of human cells[12], killing and photoproduct formation in Chinese hamster cells[13], killing, transformation and photoproduct formation in Syrian hamster cells[14], mutations in mouse cells[15] and killing and photoproduct formation in frog cells[9]. These data are summarized in Fig. 2. It is remarkable that the data points obtained for these different endpoints, by a number of different experimenters, cluster together. Above 280 nm they lie parallel to the action spectrum obtained for affecting bacterial and viral DNA. Thus, these points define the best mammalian DNA action spectrum available at present.

The action spectra shown in Fig. 2 deviate systematically from the absorption spectrum of DNA at 270 nm and at 313 nm. The high values for the action spectrum points at the lower wavelength are surprising and, at least for photoproduct formation, are not repeatable by others[17]. I suspect that there is probably a systematic dosimetry error involved. At 313 nm the difference between the absorption and action spectrum is explicable if one remembers that at this long wavelength the absorption coefficients of the

individual bases of DNA are very low and there is no tight coupling or energy transfer among them as there is at shorter wavelengths. At long wavelengths most of the absorption arises from the guanine residues in DNA, but the action arises mostly from effects on pyrimidines. Hence, the difference between absorption and action spectra at 313 nm is a special case of a more general proposition that action and absorption spectra will not coincide in multi-component systems.

If skin cancer really arises from the effects of UV-B on DNA, the action spectrum for affecting DNA shown in Fig. 2 is not the proper one to use because the sensitive targets in skin are not at its surface but the light reaching them is filtered by the upper layers of skin. The action spectrum must be multiplied by the appropriate transmission spectrum of skin[11]. An average DNA action spectrum filtered by skin is shown in Fig. 3 (ref. 11,18). The action spectrum for affecting DNA differs significantly from that for erythema and differs extensively from that for the response of the Robertson-Berger meter (Fig. 3). Thus a 1% change in ozone will result in an approximate 2.3% change in damaging UV corresponding to a DNA action spectrum, a 1.7% change corresponding to an erythemal action spectrum and an approximate 0.8% change for a spectrum corresponding to the RB meter[19].

IMMUNE EFFECTS

Carcinogenesis is a complicated process and involves much more than only the initiation phase which seems to be associated with DNA damage. Other important aspects of carcinogenesis are those of promotion and immunosurveillance. There are no good data indicating which are the rate-

limiting steps for skin cancer induction -- initiation, promotion or immunosurveillance. The last is important because of the clear evidence that UV-B, at erythemal or suberythemal levels, affects immune competence[1]. In particular, UV irradiation of mice inhibits the ability of animals to reject tumor cells transplanted from UV irradiated animals[20]. A photobiologically analogous effect is the suppression of contact sensitivity by the irradiation of mice[21]. These suppressions of an immunological response are not confined solely to the region irradiated and hence are systemic effects. The suppression of contact sensitivity takes place at low doses and, hence, it has been possible to determine an action spectrum for it. Such a spectrum is shown in Fig. 4. It differs markedly from the DNA absorption spectrum, and its relatively high values at 320 nm compared to the DNA action spectrum (see Fig. 3) indicates that ozone depletion would have a much smaller effect on this biological endpoint than on DNA damage. A difficulty with the interpretation of such experiments is that they are normalized to a value of 1 at 270 nm. If the target for the effect is well below the surface of the skin, the points at short wavelengths are much too low, and the proper normalization in terms of dose at the level of the target cells could change the entire shape of the action spectrum so that the true points at long wavelengths should be extensively lowered compared to those shown in Fig 4. The magnitude of such a correction is not known, but nevertheless, the data at the long wavelengths shown a decrease with wavelength appreciably less than that of the DNA action spectrum, supporting the conclusion that the action spectrum for immunosuppression is very different from that affecting DNA. Hence, an important experimental problem to be solved, if one is to make good

predictions for skin cancer incidence resulting from ozone depletion, is the nature of the rate-limiting steps in the carcinogenesis process -- initiation by presumably DNA damage, or immunosurveillance, or promotion (action spectrum unknown).

PREDICTIONS OF SKIN CANCER INCIDENCE RESULTING FROM OZONE DEPLETION

The data on the incidence of nonmelanoma skin cancer in the United States obtained by the U. S. National Cancer Institute[23] show clear trends as a function of latitude or relative UV-B exposure at different places in the United States. If one is to predict the change in incidence as a function of change of ozone concentration, one must disentangle the many variables involved in comparing the populations at different locations. Some of these variables are shown in Table 1.

TABLE 1. Some variables in sunlight-induced skin cancer

I:	UV irradiance averaged over the year	Life style
T:	Time of day at which exposure begins	
t:	Time duration per exposure	
n:	Number of exposures per year	
A:	Age	Sampling factors
G:	Genetic background	
O:	Occupation	
E:	Other environmental factors (wind, visible light, temperature....)	

The only one we are concerned with is the change in ozone, but, in order to evaluate this change, we have to assume that we are able to make corrections for the other variables indicated in Table 1 or that the other variables are independent of location. This certainly is not the case. The genetic backgrounds of individuals in the north of the United States are different from those in the south; and their exposure habits, the average time of day at which exposure may begin, the number of exposures per year and the average duration per exposure, certainly differ from one location to another. Unfortunately, we lack any information at present on the values of such variables. Hence, it is customary to ignore them and to relate nonmelanoma skin cancer incidence to UV exposure on the basis of the many arguments, some of which are presented above, relating UV-B to nonmelanoma skin cancer incidence.

Figure 5 shows a comparison of age adjusted incidence data for males and females, for basal and squamous skin cancer, for the 1977-1978 survey and the 1971-1972 survey of the U. S. National Cancer Institute[1,23]. The ordinates for basal cell cancer indicate an approximate 30-40% increase from the earlier to the later survey, but no significant (5-15%) increase for squamous cell cancer. The abscissa in Fig. 5 is proportional to Robertson-Berger meter readings. Similar straight lines but with different slopes would be obtained if the abscissa were the UV-B radiation weighted by the DNA action spectrum because the intensity of wavelengths affecting DNA are strongly correlated with those of the Robertson-Berger meter. Indeed the Robertson-Berger meter readings are also correlated with visible radiation. Hence, one cannot use present epidemiological data to infer the wavelength region effective in skin cancer. One must go back to basic cellular and animal data.

The National Research Council report[1] presented two calculations of the effects of ozone depletion on skin cancer incidence. One was based on the Robertson-Berger meter readings, and the second was based on calculated UV flux affecting DNA in skin. The latter values were also corrected for various statistical biases associated with the nonuniform exposure of the population at a particular location and the nonuniform exposure over the body surface (this correction amounts to a factor of approx. 1.7). Table 2 shows a comparison of these calculations. It is apparent that the predicted increases based on the Robertson-Berger meter are less than those based on the DNA action spectrum as is to be expected in view of Fig. 3 and, as has been known for some time, the percentage increases in the south of the United States are appreciably greater than those in the north. The use of the factor for correcting for bias (see column C in Table 2) indicates that a 1% decrease in ozone concentration would give a predicted increase of 2 to 5% for basal cell cancer and from 5 to almost 10% for squamous cell cancer. These numbers agree with those from calculations based on theories that assume that the shapes of the dose-response curves for skin cancer in mice are similar to those for people and that the dose to which people are exposed is proportional to their age. Calculations based on such a theory predict an approximate 5-fold increase in nonmelanoma skin cancer for a 1% decrease in ozone concentration[24].

There are obviously many things we do not know about UV induced skin cancer. There are basic gaps in our fundamental biological knowledge. Even assuming good biological knowledge, the uncertainties in the predictions of skin cancer increases as a result of ozone depletion are probably greater than the uncertainties in the predictions of expected ozone depletions resulting from human activities.

TABLE 2. Estimates of Percentage Increases in Skin Cancer Incidence in the U.S. White Population Due to a 5% Decrease in Stratospheric Ozone

	A	B	C
Basal cell			
Minn. - St. Paul			
Male	5.6	7.7	13.0
Female	4.4	5.8	9.8
Dallas - Ft. Worth			
Male	8.4	16.7	28
Female	6.8	11.3	20
Squamous cell			
Minn. - St. Paul			
Male	8.7	12.7	24
Female	9.2	12.0	21
Dallas - Ft. Worth			
Male	13.3	26	49
Female	14	25	46

Taken from NRC, 1982 (ref. 1)

A: From R-B meter readings (Scotto, et al., 1981, ref. 23).

B: From UV-B weighted by a DNA action spectrum (E. L. Scott, personal communication).

C: Column B corrected for variations in doses among individuals and parts of the body (Scott and Straf, 1977, ref. 18).

ACKNOWLEDGMENTS

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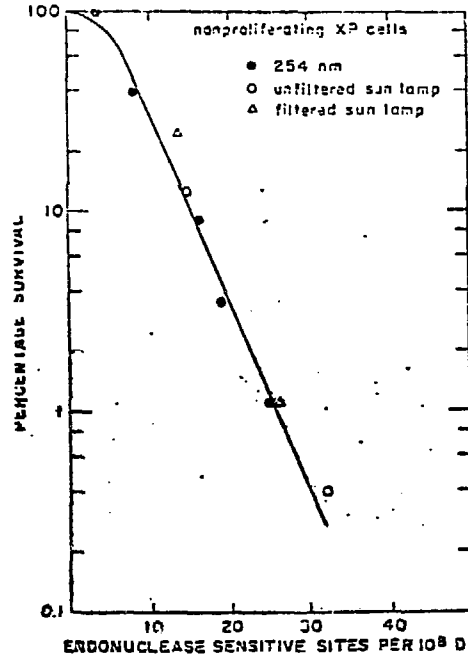

Figure 1. The survival of nonproliferating *xeroderma pigmentosum* cells as a function of the density of endonuclease sensitive sites in cellular DNA for three types of UV radiation. The filtered sunlamp contained little radiation below ~ 290 nm. From Kantor and Setlow[5].

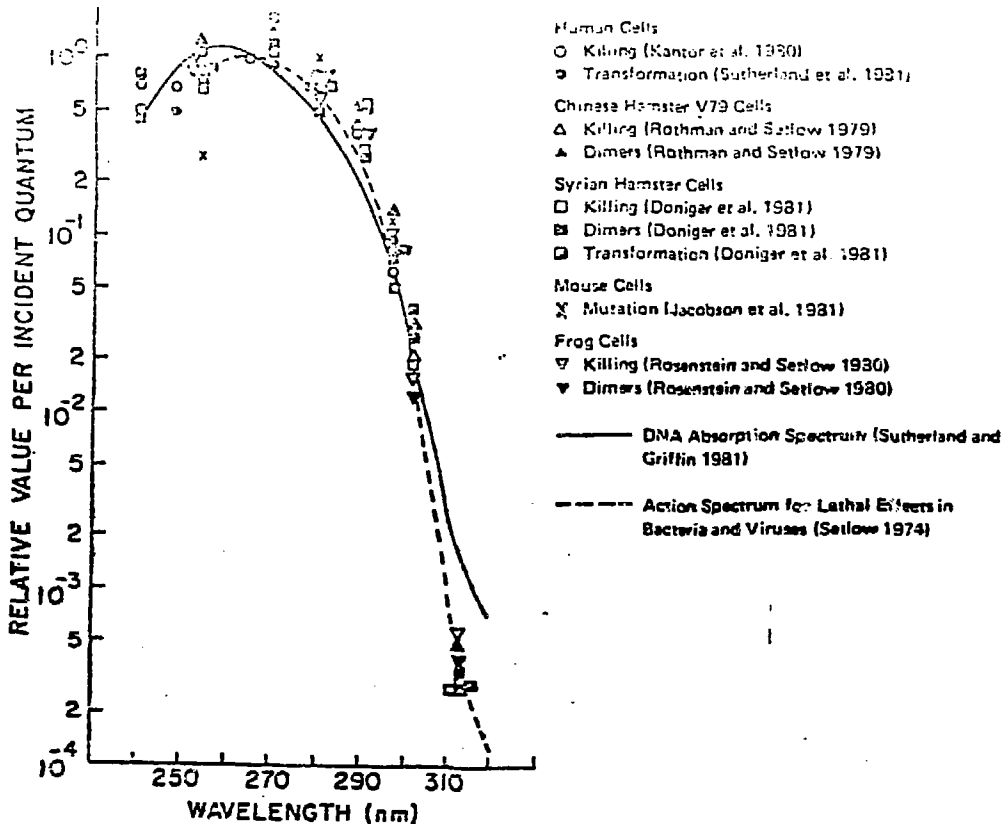

Figure 2. Action spectra points for affecting vertebrate cells. Data from the references indicated. The action spectrum for affecting prokaryotic cells[11] and the absorption spectrum of calf thymus DNA[16] are also shown.

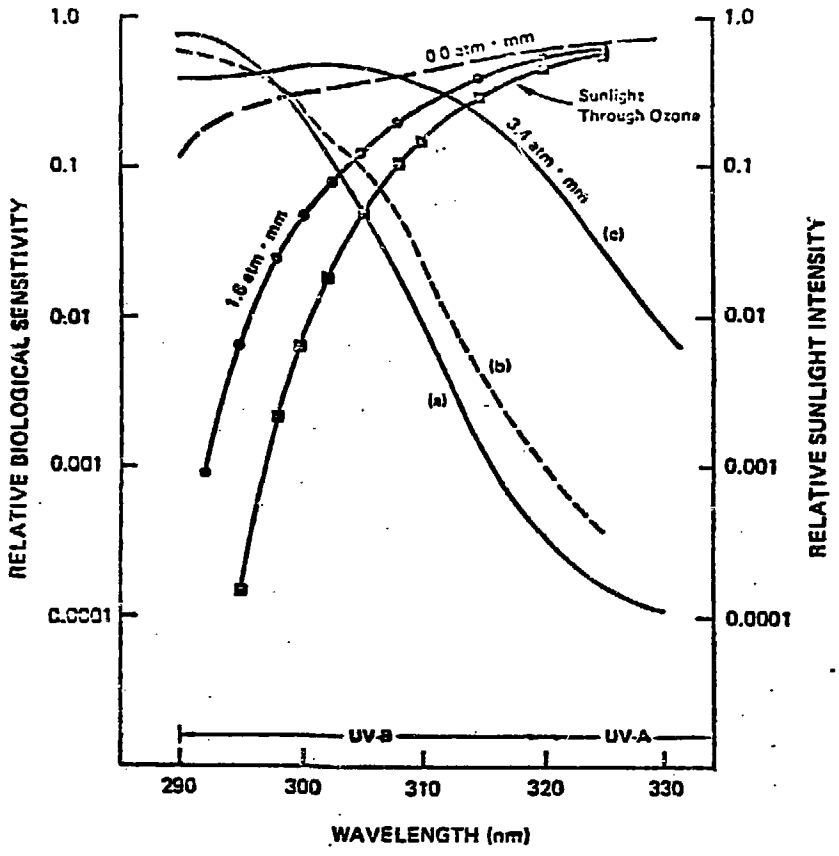

Figure 3. A comparison of sunlight reaching the surface of the earth and three often-used response spectra: (a) damage to DNA multiplied by the transmission of human epidermis; (b) human erythema; (c) Robertson-Berger meter. From ref. 1.

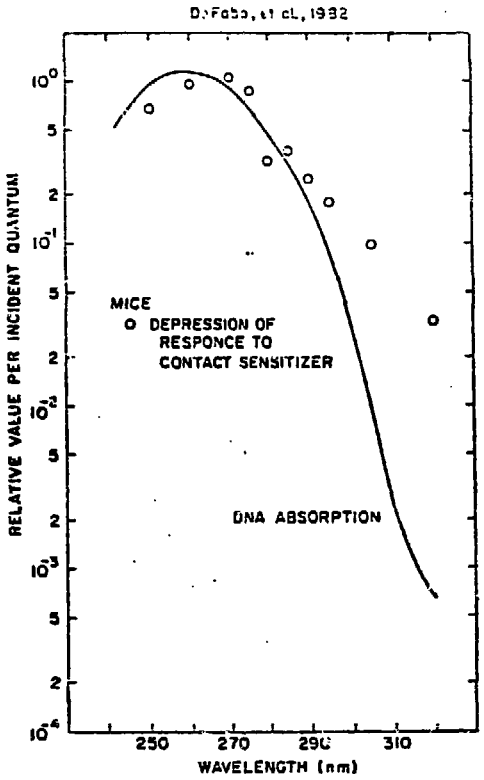

Figure 4. A comparison of the action spectrum for inhibiting the response of mice to a contact sensitizer[22] and the absorption spectrum of calf thymus DNA[16].

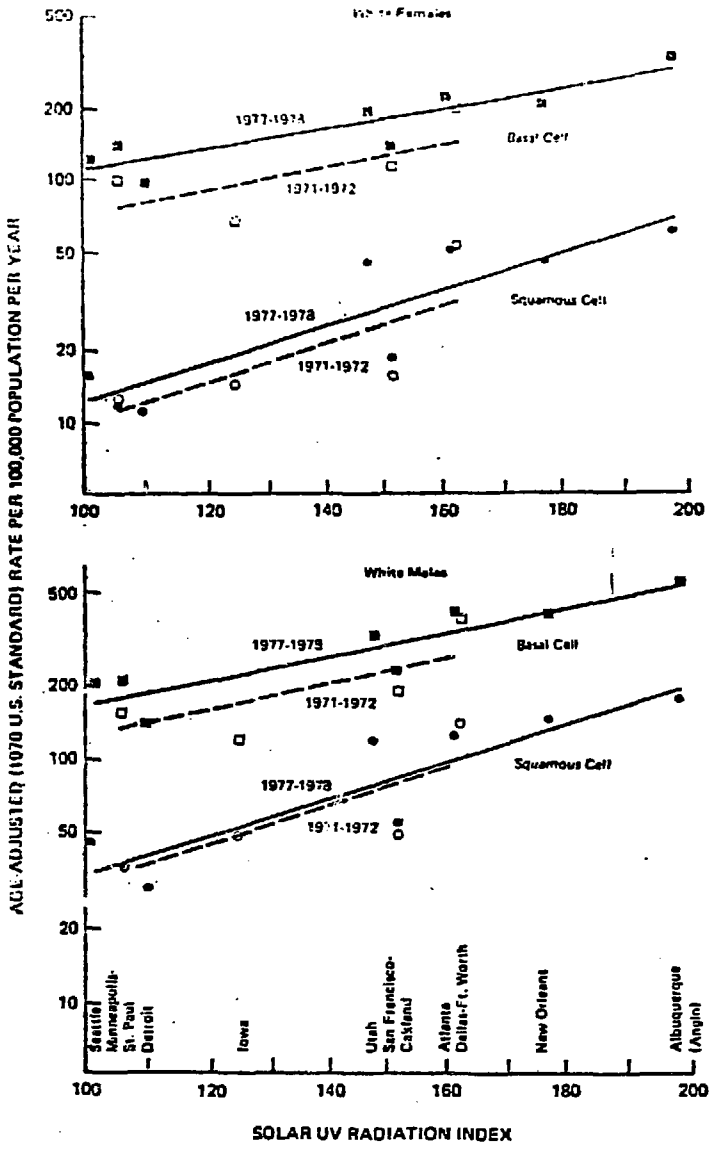

Figure 3. Age-adjusted basal and squamous cell incidence rates for U. S. Whites versus the relative Robertson-Berger meter readings (F) at a number of locations.

The model used to fit the various data sets was

ln (incidence) = $\alpha + \beta F$ where α and β are dependent on disease, sex and time of survey. From ref. 1; source of data ref. 23.