Project: "Effects of Varying Doses of UV on Mammalian Skin: Simulation of Decreasing Stratospheric Ozone."

A. Introduction:

The malignancies and premalignancies unequivocally associated with sun-exposure include solar keratoses, basal cell epitheliomas, squamous cell carcinomas and keratoacanthomas. The evidence that such tumors might be associated with solar radiation includes their increased prevalence on parts of the body habitually exposed to sunlight, in lightly pigmented and, thereby, less protected individuals in areas of the world which have the greatest insolation, and in individuals who spend more time out of doors.

The problem of ultraviolet carcinogenesis is complex. Among the factors which must be considered are (a) relative effectiveness of different wavelengths in carcinogenesis, (b) dose-response relationships, (c) time-dose effects, (d) repair processes and (e) immunologic considerations. Closely related to the above are the environmental effects on UV carcinogenesis, which involve geographic, temporal, anatomical, and life-style-related considerations (e.g. modification of the stratospheric ozone layer by human activities) This work deals with factors a-c and the ozone depletion problem explained below.

Although it is generally agreed that the UVB portion of the solar spectrum (i.e. 290-320 nm) is most effective in causing skin cancer, knowledge of the relative effectiveness of these wavelengths is not well known (i.e., reliable action spectra do not exist). Moreover, the UVA region (320-400 nm), which was long thought to be unimportant in UV carcinogenesis is now known to augment UVB sunburn response and also appears to augment the UVB carcinogenesis. In preliminary experiments, we observed that the photo-augmentation effect for sunburn response is time-dependent; the time interval between exposure must not exceed six hours for the effect to be observed. The observation of UVA involvement is of great practical (as well as mechanistic) importance because of the predominance of UVA in the total UV solar spectrum.

Because of the complexities brought on by the above-mentioned photo-augmentation phenomenon, as well as by competing repair-, biochemical, and immunologic mechanisms, the dose-response or time-dose effects may not be straight forward. Indeed, time-dose reciprocity does not exist for UV-induced tumors (Urbach et al, 1979; our own data to be published). The detailed knowledge of time-dose-response characteristics are not known, but are required for explanation and prediction of observed carcinogenic effects.

In recent years, concerns have been raised that environmental pollution may affect skin cancer incidence by affecting the stratospheric ozone shield. The ozone shield removes more than 99% of ultraviolet radiation of wavelengths less than 320 nm from the incoming sun's rays. (Emmett, 1979). The ozone is formed by the interaction of oxygen and wavelengths shorter than 240 nm. The resultant ozone then screens out longer ultraviolet wavelengths up to about 320 nm. The ozone is naturally destroyed by various mechanisms

including combination with OH ions from water and destruction catalyzed by certain gases such as NO. The stratosphere differs from the lower atmosphere (trophosphere) in that it has a very low turnover rate so that any pollution accumulates and is very slowly removed.

Man could, theoretically, reduce the stratospheric ozone concentration by a variety of ways including thermonuclear explosions, exhaust gases from SST's, and halomethanes used as spray propellants. Predictions of the degree to which these activities will increase the actual frequency are difficult to make because they require estimation of several relationships which are not at present well known, namely the degree to which these man-made activities will increase the amount of ambient UV radiation, and the degree to which these increases will be effective in producing cancer. (We already have a glimpse of the problems posed in elucidating the degree to which UVB increases will result in skin cancer, for an appreciation of the formidable problems associated with assessing the degree of ozone depletion, see J. L. Fox, Chem and Eng. News, Oct 15, 1979, pp 25-35). One estimate (T. H. Maugh II, Science, 1980, 207: 394-395) projects a 16% reduction in stratospheric ozone and consequent 44% increase in UVB radiation reaching the earth's surface.

B. Objectives of this Work:

The overall objectives addressed by these studies are of a two-pronged nature: they are concerned with (1) the effects of superimposing a constant or varying band of UVA radiation on the action of UVB light ("photoaugmentation effect") and (2) the effect of depleting the ozone layer. To accomplish this goal, we irradiated albino hairless mice with "solar-simulating" ultraviolet light and with "monochromatic" light at selected wavelengths in the UVA and UVB regions. Solar-simulating UV was obtained from a 1600 watt ozone free Xenon arc with its emission passed through a 45° dichroic mirror and a 2 mm Corning #9863 filter. This combination limits the spectral output to the range 290-400 nm. The #9863 filter does not significantly affect the short wavelength cutoff, which is limited to 290 nm by the impure quartz used for ozone-free emission. "Monochromatic" light (band width 10 nm) is isolated from a 200 watt Xenon-mercury arc (Canrad Hanovia) by means of a Bausch and Lomb high intensity grating monochromator. To simulate varying thicknesses of stratospheric ozone, we vary the amount of UVB while keeping that of UVA essentially constant. This is accomplished by filtering the solar simulating (290-400 nm) radiation through a series of Schott WG-320 filters ranging from 0.5 to 4.0 nm.[K] These are sharp cut-off filters whose characteristics are given in Table 1. UVA light is obtained by means of filtering 290-400 nm solar simulating light through a Schott WG-345 filter, which cuts off essentially all light below 324 nm (Table 1). Absorption data was obtained using a GCA-McPherson Absorption Spectrophotometer.

TABLE 1

Characteristics of Schott Filters used to Vary Amount of UVB Output from Solar Simulating Lamp.*

Filter	thickness (mm)	λ cut	λ 50%	λ 90%	λ 99%
WG-320	0.5	310	305	296	292
"	1.0	313	308	300	295
"	2.0	318	313	305	300
"	3.0	321	316	307	303
"	4.0	323	318	309	304
WG-345	2.0	346	341	330	324

*All wavelengths in nm. λ cut refers to approximate wavelength where filter starts to cut off. λ 50%, λ 90% and λ 99% refer to wavelengths where 50, 90, and 99% of the incident light is absorbed.

C. Experimental:

I. Relative Carcinogenicity of "Monochromatic" Bands of UV Radiation

Experimental: Groups of five animals were exposed to "monochromatic" light (10 nm bandpass) at 280, 301, 307, 313, and 366 nm. For the first three wavelengths, the dose was 8.9 $J \cdot cm^{-2}$ for 5 days, and then decreased to 4.5 $J \cdot cm^{-2}$. For the 366 nm irradiation, the dose was 75 $J \cdot cm^{-2}$. The same test sites were exposed to the same light for five consecutive days per week. Observations were made on a daily basis, and responses were graded on a 6-point scale as follows:

E_1 - Mild to moderate macular erythema

E_2 - Intense macular erythema

1+ - Light scaling with or without accompanying erythema

2+ - Firm, scaling, palpable keratosis

3+ - Raised, palpable keratotic plaque, corresponding to early malignant changes as defined by Epstein et al (1969)

4+ - A papilloma or tumor corresponding to extensive malignant development.

In a companion experiment, irradiation at the above wavelengths was promptly followed by 6.5 $J.cm^{-2}$ UVA light. For the 280 nm and 313 nm experiments, the fluence was 0.2 $J.cm^{-2}$ day^{-1}, for both augmentative and non-augmentative conditions. At 301 and at 307 nm, where the action is much greater, the fluence was 0.02 $J.cm^{-2}$ day^{-1} and 0.01 $J.cm^{-2}$, day^{-1} for 301 and 307 nm radiation respectively. In the latter cases, the output from the monochromator was attenuated by means of wire screens which had been calibrated with the thermopile. Precancerous responses were graded as described above. In cases where the mice burned the amount of energy required to produce each of these two types of "action" were recorded; their reciprocals were plotted as a function of excitation wavelength to yield preliminary "action spectra" for precancerous changes (minimal erythema) and burning (figures 1 and 2 respectively). In cases where burning resulted, no further irradiation was carried out. It is doubtful that either heat generated from the lamp or straight infrared radiation are the primary causes of burning since we observe a pronounced wavelength effect for the latter phenomenon (see below).

At selected intervals, mice were sacrificed and skin biopsies of the irradiated areas are taken. Histological preparations (see below) were monitored for morphologic and biochemical changes.

Results:

Precancerous lesions were induced at 280, 301, 307, and 313 nm. These same wavelengths also produced burning. No precancerous lesions were seen at 366 nm despite the much higher irradiation dosage. Both burning and E_2 responses appear to peak at 307. The shape of the two action spectra appear to differ, especially on the short wavelength side where the effects seem to fall off more rapidly in the case of the burning. Similar effects have been noted by Urbach, 1969. These results also suggest that UVA can photoaugment the effect of UVB, even though it is a poor carcinogen by itself.

Preliminary investigations reveal the following: H and E preparations show that irradiation produces marked epidermal thickening and increase keratosis. These changes are wavelength-dependent and they seem to parallel the clinical responses. However, there were some ambiguities, which probably arose from difficulties in obtaining good biopsies due to the small size of the monochromator-induced tumor. This was especially in the case where UVA radiation from the solar-simulator was used in conjunction with the monochromator.

Comment: These data, though preliminary in nature and, therefore, subject to some modification, nevertheless illustrate some important points. First, they suggest that UV carcinogenesis may be preferentially produced by light near 307 nm. It is difficult to assess the chromophore(s) responsible for precancerous lesions and/or burning from this data, especially as the observed action spectrum may be considerably distorted from the true chromophore absorption, in the short wavelength region by internal scattering and absorption by non-active chromophores. By the same token, it is difficult at this juncture to ascertain whether the apparent differences between the action spectrum for skin cancer and the action spectrum for burning arise from differential action of endogenous chromophores or whether the changes arise from early chemical modifications which affect the subsequent absorption properties of the skin for action which occurs at a later stage (burning). Further mechanistic data is needed to help resolve these questions.

II. Solar-Simulator Experiments

Experimental: While the results of the preceding experiment indicate that shorter wavelengths do not necessarily increase carcinogenesis, we feel that data using monochromatic light is "artificial" and cannot be extrapolated to environmental conditions, since sunlight emission is a polychromatic continuum. We, therefore, conducted experiments with the 1600 watt solar-simulator as described above as well as with broad band "monochromatic" radiation of UVB centered at 300 nm (half value band width = 20 nm). In order to enable a meaningful comparison between the two sources and to gauge the magnitude of UVA in augmenting the UVB effects, an "effective" dose was calculated for both sources by convoluting the action spectrum determined above with the spectral distribution of the emitting source (Willis et al, in preparation). Experiments involving both sources were carried out under conditions of a) constant irradiation ($\sim$ 1 MED) and b) a regimen whereby the dose was increased at 20% increments (of the standing dose i.e. 1 MED) after every five days of irradiation. Additional experiments were carried out with broad band UVA (solar-simulating radiation filtered through a Schott WG 345 cut-off filter). Clinical and histological responses were graded as previously described. Twenty animals were used in each experiment.

Results:

a) UVB radiation. After 30 days (total dose 1.62 J/cm^2, effective dose 1.44 J/cm^2) 75% of the animals had 1+ response, 20% had 2+, and 5% had 3+ responses. At the 3+ stage, histopathological changes were compatible with early squamous cell carcinoma, or carcinoma in situ. There were cells with large and bizarre nuclei, as well as "rounding off" of cells, with apparent loss of desmosomal attachments. In many (but not all regions, the basement membrane appeared to be intact. Since the epidermis becomes thickened on exposure to UV radiation, we felt that it should be possible to incrementally increase the dose without burning the skin. This turns out to be the case. For equivalent effective doses, the latter regimen results in increased clinical and histological severity of response. An example is given in Table II.

Table II - Comparison of Constant vs. Incrementally Increased Doses of UVB Radiation.

	Eff. Dose (J/cm^2)	1+	2+	3+
Constant Dose	1.24	15	4	1
Increased Dose	1.23	4	12	4

(See "Experimental" for detail of dosage, source, etc.)

b) UVA Radiation: Daily exposures of 62 J/cm^2 were given for 30 days at 5 days/week. By day 10, 65% of the animals exhibited 1+ responses, but this regressed until, by day 30, only E$_1$ (minimal erythema) was present.

c) Effect of UVA + UVB: Twenty mice were irradiated with a constant daily dose of 9.0 J/cm^2 ($\sim$1 MED) effective dose = 0.113 J/cm^2). At the end of 30 days (effective 3.40 J/cm^2, 80% of the mice had developed 3+ reactions, as compared to 5% for UVB alone. The effect of UVA in augmenting precancerous and carcinogenic effects of UVB is further indicated in Table III.

Table III - Photoaugmentation of UVB by UVA Radiation at Constant Dose

	Eff. Dose (J/cm^2)	1+	2+	3+
UVB	1.44	15	4	1
UVB + UVA	1.59	2	4	14

Histopathologic changes paralleled those occurring for UVB radiation alone, but were more pronounced.

As was the case for UVB radiation, when the doses were increased at 20% increments every fifth day, the response at a given dose was more severe than for the constant regimen. After 18 days, three animals (15) had developed 4+ responses (advanced tumors) after 18 days, and the number of 4+ reactions continued to increase up to day 30. At equivalent effective doses of UVB, no animals had developed 4+ reactions.

Histopathologic changes in the 4+ mice showed atypical mitotic figures, hyperplasia and hyperchromasia of cellular nuclei, disintegration of intercellular bridges, and increasing variability in all sizes. Several specimens obtained 4-6 weeks after irradiation revealed so-called "spindle-celled" squamous cell carcinomas. This type of tumor closely rsembled a fibrosarcoma with spindle-shaped cells extending from the epidermis to deep into the dermis. This unexpected histopathological finding is of extreme interest, since it is the type reported to occur in areas of radiodermatitis in humans, and is regarded as a relatively rare Grade-4 highly malignant metastatizing form of squamous cell cancer.

Comment: These results indicate two major points: Firstly, time-dose reciprocity does not exist, as evidenced by comparison of responses at equivalent effective doses of either UVB or UVA + UVB radiation (Table II). Secondly, despite the relatively poor carcinogenic effectiveness of UVA radiation, it can augment the carcinogenic effects of UVB, as evidenced by the comparison in Table III. This demonstrates that UVA effects must be explicitly considered when effects such as ozone depletion (see below) are considered.

III. Simulation of Ozone Depletion

Experimental: Schott WG-320 filters (Table I) were used in conjunction with the solar-simulator, as described above, to produce conditions aimed at mimicing the effect of varying ozone layer thickness. The spectral distribution of the solar-simulator under the various conditions was measured. The wavelength at which the output is down to 1% of its value at

340 (plateau region) is given in Table IV. This wavelength is somewhat arbitrarily defined as a cutoff wavelength.

TABLE IV

Cutoff Wavelength of Solar-Simulator/WG 320 Filter Combination - Minimal Erythemal Dose (MED) for Each System.

Filter (mm)	Cutoff Wavelength (nm)*	MED (J/cm^2)
None	301.0	7.05
0.5	303.5	13.4
1.0	305.5	17.3
2.0	307.5	22.4
3.0	308.5	25.7

*Wavelength where output is 1% of that at 340 nm.

Groups of 10 animals were exposed to the light from the solar-simulator which had been filtered with the various Schott filters (including no filter). Minimal erythemal doses (MED) were determined for each set of conditions. Following MED determination (Table IV) two sets of experiments were carried out, using the technique of incremental increases described above. Firstly, equal doses of radiation (0.9 MED for the "no filter" condition) were used for each filter combination. In the second set of experiments, doses equivalent to equal responses (i.e. 0.9 MED for each filter combination) were used. Responses were evaluated as previously described.

Results:

a) Constant Dose: Only the "no filter" and the 0.5 mm filter combinations produced lasting precancerous changes beyond the E_2 stage. These changes were along the lines of those described previously. The effects due to 0, 2.0 and 3.0 mm combinations eventually regressed back to the E_1 stage or they disappeared altogether.

b) Dose Equivalent to Constant Biologic Effect: Much different results were obtained for two groups (of 10 and 8 mice respectively per filter combination) which were irradiated with light equivalent to 0.9 MED at each filter combination. In this case, the "no filter" group clearly progressed to the 3+ (early cancer) stage after 30 irradiation days, whereas the remaining mice all exhibited effects which were difficult to distinguish from one another (mostly 2+ responses, some 3+ with or without moderate burning).

Comment: These experiments disclose several salient features. Firstly, they again attest to the superiority of the UVB component in causing pre-cancerous and cancerous changes. The results again point out the non-reciprocal time-dose relationship. Indeed, it appears that in choosing our experimental conditions, we may have chosen two extreme cases - one (equal dose) in which little or no net response is observed and the other (equal response) in which the precancerous response in each system (obscured somewhat by burning) are relatively acute and difficult to tell apart. In order to obtain data which would allow a more quantitative distribution of these wavelength effects, it appears that some intermediate condition(s) for irradiation will have to be carried out. Conversely, these results also imply that observed increases in skin cancer may not be related to ozone depletion in a straight forward way: the effects of dose, angle, environmental and other factors previously mentioned will all influence the results inasmuch as they are determinants of solar dose and spectral distribution as functions of time.

General Prognosis: The above results indicate the complex nature of the problems of explaining and predicting the effect of broad band solar UV light in producing carcinogenesis, and of predicting the effects of ozone depletion on these processes. The combined effect of component wavelengths in polychromatic UVA + UVB light is different from the sum of its individual components, and the augmentation process must be better understood. Hence, reliable action spectra for UVB carcinogenesis in the presence and absence of UVA are needed, and such work is projected for the future. Since time-dose reciprocity does not hold, the provision of a much more detailed picture of the dose-response characteristics under different filtering conditions (i.e. "different ozone concentrations") is also projected. The overall aim is to eventually explain and predict the observed results on a more basic level. Therefore, future studies will involve the expansion of the scope of histological, biochemical experiments and to conduct mechanistic photochemical studies on the molecular level.

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