[CANCER RESEARCH 47, 1825–1829, April 1, 1987]

Action Spectra for Human Skin Cells: Estimates of the Relative Cytotoxicity of the Middle Ultraviolet, Near Ultraviolet, and Violet Regions of Sunlight on Epidermal Keratinocytes[1]

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ABSTRACT

Action spectra for the cytotoxic action of electromagnetic radiation in the solar range 280–434 nm have been determined for human fibroblasts and epidermal keratinocytes derived from the same foreskin biopsy. The spectra for the two cell types are close to identical and coincide with our previously published data for a human lymphoblastoid line indicating that the mechanism of inactivation of the three human cell types is similar at any given wavelength. Using published data for ultraviolet transmission of human skin and sample spectral irradiance data, we have estimated the relative biological effectiveness of the middle ultraviolet (UVB) (290–320 nm), near ultraviolet (UVA) (320–380 nm), and violet (380–434 nm) regions of sunlight for cytotoxicity at the basal layer of the epidermis. We conclude that the UVB component in noon summer sunlight (the most UVB rich spectral conditions tested) may contribute only about 40% of the total cytotoxic effectiveness of sunlight at 290–434 nm. At lower zenith angles, UVA can account for up to 80% of the cytotoxic effectiveness of the combined UVA and UVB regions. Finally, a comparison of published action spectra data for human erythema with cytotoxicity data corrected for ultraviolet transmission to different depths of the human epidermis suggests that UVA erythema could be causally related to cytotoxicity occurring at an average depth of 40–50 μm into the human epidermis.

INTRODUCTION

Photochemical data obtained in bacteria have provided useful data for the prediction of the important wavelengths in sunlight that lead to genetic damage and eventually skin cancer in humans (1). However, the mechanisms for processing UV radiation induced damage differ considerably between bacteria and cultured animal cells (2) so that action spectra for such parameters as cytotoxicity and mutagenesis would be expected to differ quite markedly between enteric bacteria (3) and human cells. Indeed, human cells (4–6) appear to be an order of magnitude more sensitive to the lethal effects of UVA[2] radiation than are bacteria (3). A similar pattern appears to be true for Chinese hamster cells (7). At the present time, action spectra for cytotoxicity to human cells are available out to 365 nm in human fibroblasts (6) and out to 434 nm in a human lymphoblastoid line (4). Recently, we have obtained a series of paired lines of human fibroblasts and epidermal keratinocytes from human foreskin biopsies (see Ref. 8). Since UVA (as well as UVB) radiations damage the epidermis (9) and most skin cancers arise from epithelial cells, the question arises as to whether fibroblasts and keratinocytes display similar sensitivities to radiation at defined wavelengths. In the following study, action spectra for the two cell types out to a wavelength of 434 nm are described. Sample solar spectral data have been used to show that UVA radiation may make a major contribution to cytotoxicity under *in vivo* conditions. In addition we have used published human skin transmission data and erythema data in an attempt to assess the role of cytotoxicity to epidermal keratinocytes in human erythema.

MATERIALS AND METHODS

Cell Strains and Culture. The human epidermal keratinocyte (EK4 keratinocyte) and fibroblast (EK4 fibroblast) lines were derived from human foreskin biopsy material and cultured as described previously (8) using a modification of a methodology originally described by Rheinwald and Green (10). Fibroblasts (plated on γ-irradiated human fibroblast cells of the same strain) and keratinocytes (plated on γ-irradiated Swiss 3T3 cells) in passages 4–6 gave cloning efficiencies of 40–50 and 3–5%, respectively. Paired lines from independent biopsies gave similar results.

Irradiation and Dosimetry Procedures. Monochromatic radiation was obtained from a 2.5-KW mercury-xenon lamp (Hanovia) in combination with a single or tandem Schoefel monochromator(s). Details of light filtration and band widths at each wavelength and dosimetry have been described previously (4). Cells of both types were irradiated in phosphate buffered saline at 4°C in suspension, a procedure which did not lead to significant cell death in the controls during the irradiation times used.

Spectroradiometric Measurements of Sunlight. The solar irradiance measurements were made (by Dr. R. D. Ley and Lee Applegate) in summertime in Albuquerque, NM, using an Optronic Laboratories Model 742 spectroradiometer.

RESULTS

Determination of Action Spectra. The comparative fluence dependent inactivation of fibroblasts and epidermal keratinocytes is shown for a series of wavelengths from 254 to 434 nm in Fig. 1 (*A–H*). The radiation sensitivity of the two cell types is similar throughout the wavelength range tested. However, the keratinocytes are consistently slightly more resistant in the UVC and UVB ranges. A marked difference is again seen at 405 nm where the keratinocyte populations appear to be twice as resistant as the fibroblast populations to radiation inactivation.

Since the data are not adequate to calculate final slopes at the longer wavelengths, the F_{10} has been taken as the parameter for the action spectrum. In a previous study, we obtained a similarly shaped action spectrum whether F_{10} or slope values were taken (4). The F_{10} values have been quantum corrected and plotted relative to those obtained at a wavelength of 254 nm on Fig. 2. As expected from the raw data the shapes of the action spectra for the two human skin cell types are extremely similar. The data point at 405 nm for the epidermal keratinocytes was obtained by extrapolation and must be considered with appropriate caution. Data previously obtained with a human lymphoblastoid line in this laboratory (4) have also been added to Fig. 2 and except at 434 nm lie close to the spectrum for the other two cell types. For easy reference we have added data obtained in two other laboratories (Refs. 6 and 12) at 313

Received 9/29/86; revised 12/31/86; accepted 1/7/87.

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[1] This investigation was supported by grants from the Swiss National Science Foundation (3.108.85) and Swiss League against Cancer.

[2] The abbreviations used are: UVA, near ultraviolet (320–380 nm); UVB, middle ultraviolet (290–320 nm); F_{10}, reciprocal of the fluence to reduce the cell population to 10%.

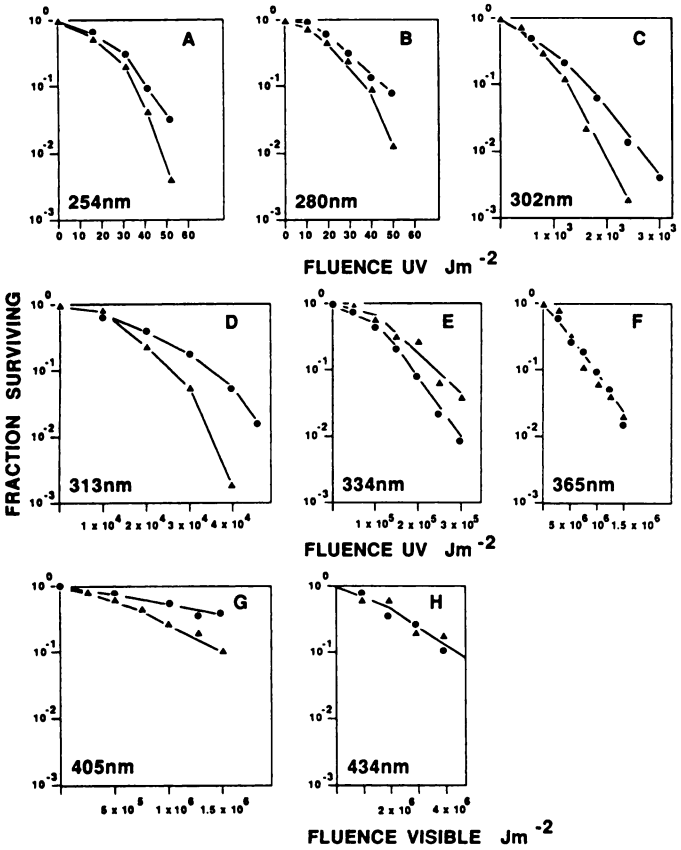

Fig. 1. Inactivation of clone-forming ability of populations of human fibroblasts (▲) and epidermal keratinocytes (●) at a series of wavelengths ranging from 254 to 434 nm.

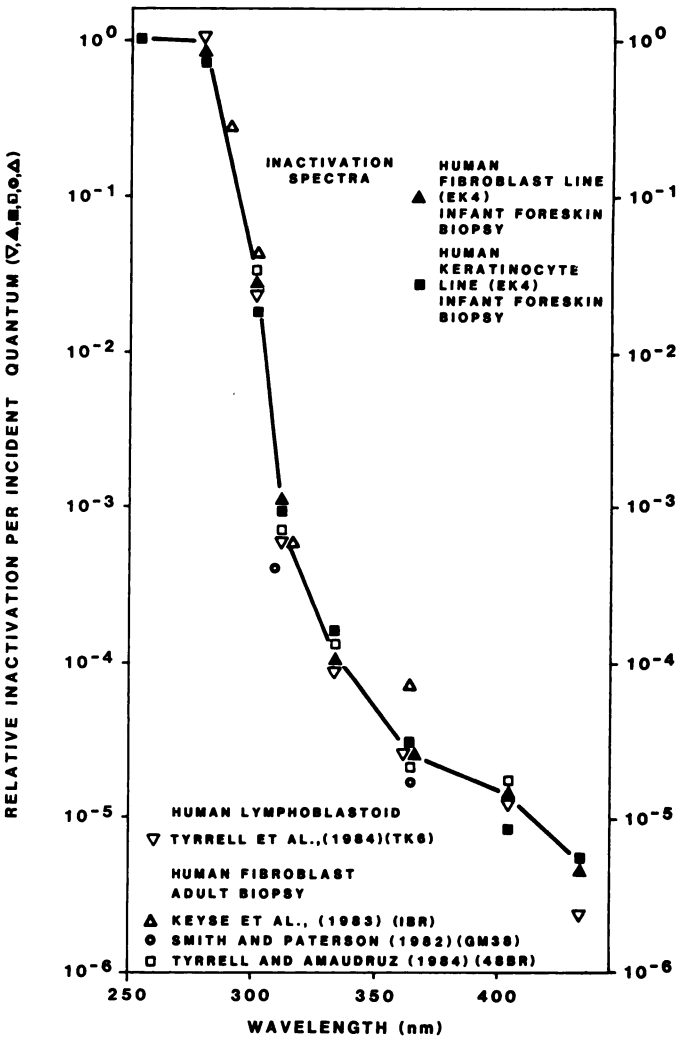

Fig. 2. Quantum corrected action spectra for inactivation of human fibroblasts and epidermal keratinocytes. The human lymphoblastoid (TK6) and one set of adult biopsy fibroblast (48BR) data are taken from previous publications from this laboratory (see Refs. 4 and 11). The adult fibroblast biopsy data (IBR and GM38) are taken from publications from independent laboratories (Refs. 6 and 12).

nm and 365 nm for human fibroblast lines. The reason for the marked divergence between laboratories at 365 nm is not clear but since data obtained with 3 human cell types in a single laboratory show a close to superimposable pattern in this wavelength region, we suspect that the differences arise from interlaboratory variation in dosimetry.

Relative Cytotoxic Effectiveness of Sunlight. The data obtained in human keratinocytes allow us to make an estimate of the cytotoxic effectiveness of sunlight at the basal layer of the epidermis as a function of wavelength. The relative biological effectiveness of sunlight at each wavelength should be given by the product of the sensitivity of the epidermal keratinocyte populations (not quantum corrected), the transmission of light through the epidermis, and the incident solar energy (under given conditions) at each wavelength. Human skin transmission data have been taken from a publication by Bruls *et al.* (13). For simplicity, we have used the average values they calculated for transmission through a 70-μm epidermis (Fig. 3). The models which are currently available for calculating solar irradiance at given geographical locations, solar zenith angles, atmospheric conditions, etc. (14), do not extend far enough into the UVA and visible ranges. We have therefore used raw spectral data (kindly provided by R. D. Ley) at a given geographical location in a Northern latitude for the data and calculations in Figs. 3 and 4, and Table 1. The values taken at 1 p.m. in mid-May (1986) are shown on Fig. 3 together with the skin transmission data and the data for the relative inactivation of the epidermal keratinocyte populations. Also included on the figure is an estimate of the relative cytotoxic effectiveness of sunlight at the basal layer of the epidermis normalized to the peak wavelength of 306 nm. It is immediately clear that even at 1 p.m. the UVA region may contribute significantly to the induction of cytotoxic damage to the basal layer of the epidermis.

In order to examine the effect of solar zenith angle on the relative cytotoxic effectiveness of sunlight, we have used sample solar spectral data taken at different times on a clear day in midsummer. Values for skin transmission and relative sensitivity of the keratinocytes have been estimated from the type of plot shown on Fig. 3 and convoluted with spectral data (each nm to 350 nm, each 2 nm to 434 nm) to give the linear plots of cytotoxic effectiveness shown in Fig. 4. From these data, we have integrated the cytotoxic effectiveness for the different spectral regions at the different times of day and these values are shown in Table 1. Also shown are the values for data taken at 1 p.m. on May 14 (see Fig. 3) and 9 a.m. on June 5. As expected from spectral data, the relative cytotoxic effectiveness of the UVA region increases markedly at higher zenith angles so that early and late in the day the ratio of UVA:UVA + UVB effectiveness is close to 4:1.

Relationship between Erythema and Cytotoxicity to Human Epidermal Keratinocytes. In view of the possible relationship between cytotoxicity in the epidermis and erythema, we have compared our spectral inactivation data with recent erythema

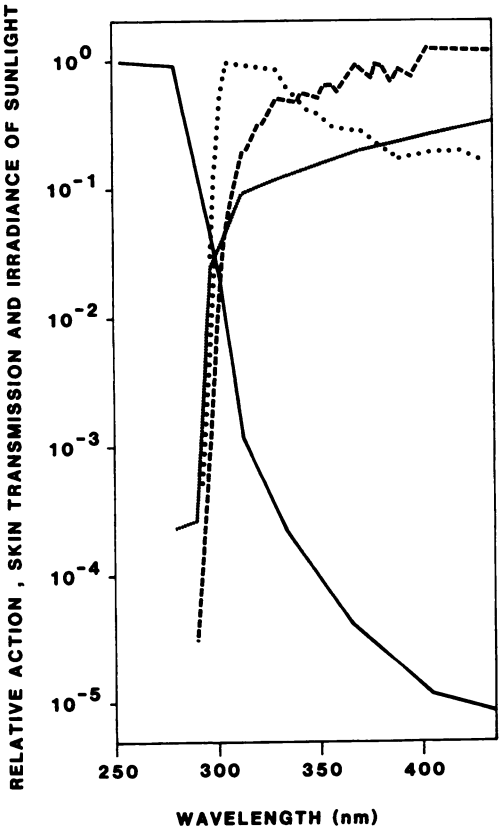

Fig. 3. Comparison of action spectra (not quantum corrected) taken from the $1/F_{10}$ values of epidermal keratinocytes expressed relative to the value at 254 nm (——) and the relative cytotoxic effectiveness of sunlight at the basal layer of the epidermis under defined solar spectral conditions (·····; for calculations and conditions see text). Also shown are sample solar spectral irradiance data (– – – –, Albuquerque, NM, 1 p.m., May 14, 1986) and average values for the fraction of light transmitted through a 70-μm epidermis (• • • • •).

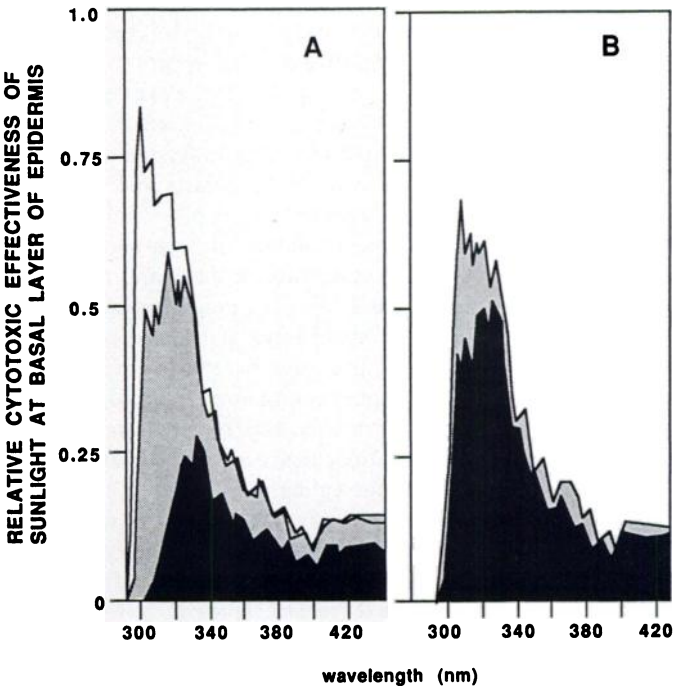

Fig. 4. Relative cytotoxic effectiveness of sunlight at the basal layer of the epidermis calculated from solar spectral irradiance data taken at different times on a single day (Albuquerque, NM, July 3, 1986). For calculations see text. *A*, 8 a.m. (*dark shade*), 10 a.m. (*gray shade*), and 12 noon (*unshaded*). *B*, 2 p.m. (*gray shade*) and 4 p.m. (*dark shade*).

Table 1 *Relative cytotoxic effectiveness[a] of the UVB (290–320 nm), UVA (320–380 nm), and violet (380–434 nm) regions of sunlight to human epidermal keratinocytes at the basal layer*

Solar conditions[b]	Total cytotoxic effectiveness (290–434 nm) relative to 12 noon, July 3	Ratio UVB:UVA: violet	UVA:UVA + UVB	Wavelength(s) of peak effectiveness (nm)
8 a.m., July 3	0.42	16:57:27	0.82	320–332
10 a.m., July 3	0.86	29:51:20	0.63	315–330
12 noon, July 3	1.0	39:45:16	0.54	305
2 p.m., July 3	0.91	31:50:19	0.61	306
4 p.m., July 3	0.72	27:54:19	0.79	310–330
1 p.m., May 14		31:50:19	0.62	306
9 a.m., June 5		27:54:19	0.75	320–330

[a] As estimated from *in vitro* data and light transmission measurements through skin (Figs. 3 and 4).
[b] All spectroradiometric measurements of sunlight taken in Albuquerque, NM, in summer 1986.

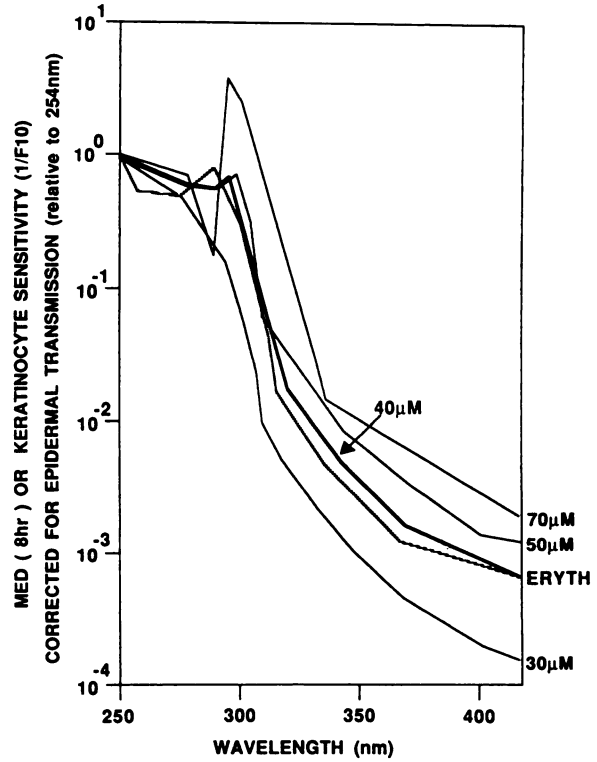

Fig. 5. Comparison of an action spectrum for human erythema and action spectra for inactivation of human epidermal keratinocytes (not quantum corrected) corrected for UV and visible attenuation at depths of 30, 40, 50 and 70 μm into the human epidermis. The erythema spectrum is shown by the *dashed line* and the estimated inactivation spectrum at 40 μm is shown by a *bolder continuous line* than for the other epidermal depths.

data from the group of Parrish *et al.* (15). Their data (from Table 1, 8-h minimal erythema dose) are plotted in Fig. 5 together with our action spectrum for cytotoxicity of epidermal keratinocytes corrected for transmission to either a 30-, 40-, 50-, or 70-μm depth of the epidermis (see Table 4 in Ref. 13). Of necessity, we can only estimate transmission data between their average calculated data points but this would appear to be the best data currently available for such an estimation. The correspondence between the erythema spectrum and data calculated for a 30- or 70-μm (basal layer) epidermal thickness is extremely poor. However the plots do clearly demonstrate how the optical transmission properties of skin enhance the UVB peak as the depth increases. The correspondence between the spectra at 40 and 50 μm is much closer and at 40 μm the curves in the UVA and nearvisible regions are close to superimposable.

DISCUSSION

The action spectra data reported herein (Figs. 1 and 2) consolidate results previously obtained from other laboratories and our own and extend the data with human skin cells out to a wavelength of 434 nm. To our knowledge this is the first action spectrum obtained using human epidermal keratinocytes (but see abstract of Ref. 16). It is therefore important to note the marked similarity between the pattern of the spectra for the different human cell types. This should enhance confidence that results obtained from radiation studies carried out with the easier-to-handle human fibroblast cell system may be applied to predictions concerning keratinocytes. The spectra also correspond reasonably well with the more limited lethal action spectra for killing of Chinese hamster cells determined by Wells and Han (7). However, the mammalian cell lines appear to be at least an order of magnitude more sensitive to UVA than the various bacterial species for which complete spectra are available in this region (see Ref. 3). This large difference between cell types may be related to the protective effect of the growth delay induced in bacteria by UVA as a result of the photolability of 5-thiouracil-containing transfer RNA and the consequent triggering of the stringent response (for review see Ref. 17). Human cells are not susceptible to this pathway and do not demonstrate significant growth delays in the sublethal fluence range.[3]

The skin transmission data used for convoluting the action spectra to calculate biological effectiveness (Fig. 3) are, of necessity, average values. However, they are derived from the most extensive and accurate set of measurements so far available for transmission through human skin. The cytotoxicity data (Figs. 1 and 2) have been derived *in vitro* and therefore may not represent the *in vivo* situation with complete accuracy. We therefore make the assumption that the intracellular milieu does not dramatically change the pattern of relative wavelength dependent sensitivity. Finally, although lethal wavelength interactions are much smaller in human cells (4) than in bacteria (18), there is an overall tendency for the longer UVA wavelengths to sensitize to radiation at the UVB wavelengths. We have not attempted to take this into consideration in the calculations of biological effectiveness in view of the lack of detailed information in human skin cells.

With these limitations in mind, we have estimated the relative biological effectiveness at the basal layer of the human epidermis under different spectral conditions (Figs. 3 and 4; Table 1). It is immediately clear that even under conditions where the UVB:UVA ratio is highest (*i.e.*, 10 a.m.–2 p.m., low zenith angle), the UVA region still accounts for 50 to 60% of the total cytotoxic damage contributed by the UVA and UVB regions of sunlight (Table 1). If the effectiveness of the entire wavelength region from 290–434 nm is integrated, Table 1 shows that the UVB region contributes only 39% of the cytotoxic effectiveness under the most UVB rich spectral conditions examined (*i.e.*, noon, midsummer, Northern latitude). The contribution of UVB is predictably even smaller under irradiance conditions of higher solar zenith angle, being 2 or 3 times lower than the UVA component in the early morning and late afternoon. The influence of UVA will be even greater if lethal sensitization by longer wavelengths occurs in human skin cells (see above).

In order to gain possible clues as to the role of cytotoxic effects on keratinocytes in human erythema, we may compare our calculated values for UVA induced cytotoxic effectiveness at the basal layer (Table 1) with those for erythemal effectiveness in humans by Parrish *et al.* (9). These authors concluded that although UVA is much less erythemogenically effective than UVB, the longer wavelength radiation may contribute around 15% of the total effect around noon and much higher values as solar zenith angle increases. We have explored the relationship between cytoxicity and erythema further by comparing our data with more recent data from Parrish *et al.* (15). The data in Fig. 5 suggest that the correspondence between spectra is closest when we use skin transmission data derived at an epidermal thickness of 40–50 μm. The close correspondence in the UVA region at 40 μm thickness (approximately the distance to the center of the average 70 μm thick human epidermis) suggests that UVA erythema could be due to generalized cytotoxic effects throughout the living epidermis. However, the peak for cytotoxic effectiveness in the UVB region around 295–300 nm appears pronounced only as transmission data for optical depths closer to the basal layer are considered. Thus, as discussed previously (15, 19), there may be more than one mechanism of sunlight-induced erythema (although both may be related to cytotoxicity). We may speculate that in the UVB region, damage to DNA is the predominant mechanism (see also Fig. 1). Indeed a DNA target for UVB-induced erythema is now strongly supported by photoreactivation experiments in animals (20). In the UVA region, cytotoxicity may well be causally related to erythema but there is little evidence that a DNA target is involved.

A final comment is related to the carcinogenic effectiveness of sunlight. Recent experiments in mice now strongly suggest that the UVA region may contribute negligibly to carcinogenic effectiveness (21). In view of the role of UVA in cytotoxicity and erythema discussed above, this could indicate that the target for erythema is quite different from the target for initiating the carcinogenic process. However, if UVB and UVA induced erythema results from distinct pathways, then it remains possible that erythema and initiation are induced through a common target (presumably DNA) in the UVB region. A clearer understanding of this relationship should soon be available from tumor photoreactivation experiments now in progress in marsupials.[4] The role of UVA in promotion (for example by the extensive repopulation that will occur after acute cytotoxic sunlight injury) remains to be thoroughly evaluated.

In summary, we have shown that fibroblasts and epidermal keratinocytes derived from human skin display similar action spectra for cytotoxicity. By convolution of solar spectral irradiance measurements, skin transmission data, and the action spectra data we predict that UVA may play a significant role in cytotoxic damage to the basal layer of the epidermis after exposure to noon sunlight and may be the major cytotoxic component of sunlight at higher zenith angles. Finally, we have an indication that UVA erythema may result from cytotoxic damage to epidermal keratinocytes occurring at an average distance of 40–50 μm into the epidermis.

ACKNOWLEDGMENTS

We are grateful to Dr. Walser and Dr. Frenck of the Dermatology Department of the Centre Hospitalier Vaudois for their efforts in coordinating the collection and initial handling of the foreskin biopsies. We also offer our sincere thanks to Dr. R. D. Ley and Lee Applegate for their generosity in carrying out the spectroradiometric measurements of sunlight especially for this project.

[3] Unpublished observations.

[4] R. D. Ley, personal communication.

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Cancer Res 1987;47:1825-1829.