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Ultraviolet light action spectra for neoplastic transformation and lethality of Syrian hamster embryo cells correlate with spectrum for pyrimidine dimer formation in cellular DNA

(human skin cancer/mutagenesis)

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ABSTRACT **Action spectra were determined for neoplastic transformation, production of pyrimidine dimers, and lethality in Syrian hamster embryo cells. Of wavelengths between 240 and 313 nm, the most effective were 265 and 270. The relative sensitivities per quantum for transformation, pyrimidine dimer production, and lethality were essentially the same at each of the wavelengths tested. This action spectrum for transformation, which is relevant to carcinogenesis, is similar to spectra obtained previously by measuring other cellular responses in either microbial or mammalian systems. Because the action spectra for cytotoxicity and transformation are the same as the spectrum for dimer production, DNA is suggested as the target for all these processes.**

The positive relationship between exposure to sunlight and incidence of skin cancer in humans has been well established (1–3). Photocarcinogenesis studies utilizing albino mice have shown 300–320 nm to be the most effective range for producing tumors in mice (4). Wavelengths shorter than 300 nm are less effective in inducing tumors because they are absorbed by the upper layers of the epidermis.

The spectral irradiance of sunlight at the earth's surface is essentially constant at wavelengths longer than 330 nm and decreases by more than 4 orders of magnitude between 330 nm and 290 nm (5). The precise spectrum for a given place and time depends on the angle of incidence of sunlight, the meteorological conditions, and the thickness of the ozone layer. Environmental factors that decrease the ozone layer can increase the intensity of wavelengths shorter than 330 nm at the earth's surface and consequently could increase the incidence of skin cancer in man. A theoretical analysis of the wavelengths in sunlight effective in producing skin cancer was developed by Setlow (6) to correlate the possible effects of changing the ozone layer on the incidence of skin cancer. An average action spectrum for effects of UV on bacterial systems (see below) was used as an estimate in Setlow's analysis because no action spectrum for carcinogenesis was available. An action spectrum for transformation utilizing mammalian cells would eliminate the need to extrapolate from bacterial models.

In early studies, the relationship between the biological and biochemical effects of UV were pursued by using microbial systems. Pyrimidine dimers, the major UV-induced photoproduct in DNA, are considered to be responsible for most of the biological effects such as cell lethality and mutation (7). Studies of UV action spectra were instrumental in implicating nucleic acid as the chromophore for killing bacteria (8, 9) and bacteriophages (10) and for mutation in fungi (11). DNA was further implicated as the target for lethality and mutation because the action spectrum for pyrimidine dimer formation (12, 13) was found to be similar to that for killing.

The *in vitro* transformation of Syrian hamster embryo cells (HEC) by carcinogens is a model system for studying carcinogenesis in a quantitative manner (14). DiPaolo and Donovan (15) have shown that morphological transformation is induced in HEC in a dose-dependent manner by UV irradiation from a germicidal lamp. More than 85% of the light emitted by germicidal lamps is at 254 nm, a wavelength highly effective in causing changes in DNA. Furthermore, cells derived from UV-induced transformed colonies produce progressively growing tumors when injected into *nude* mice (16). Therefore, the HEC transformation system is relevant for a determination of the action spectrum of carcinogenesis. Unlike the *in vivo* situation, no epidermal layer screens the target cells from receiving the incident UV light.

In this study, the action spectra for transformation, lethality, and pyrimidine dimer production in the cellular DNA were determined concurrently. These three action spectra were similar to one another and to those previously determined for bacterial systems.

MATERIALS AND METHODS

Cells, Transformation, and Survival Assays. Fresh secondary HEC were used in all experiments except as noted. Cells were cultured in Dulbecco's modified Eagle's medium supplemented with 15% fetal calf serum (hereafter referred to as "culture medium") and incubated in an 11% CO_2 humidified atmosphere at 37°C. Specific details of the transformation and cell survival assays have been presented (16). Cells were UV irradiated (see below) 1 day after they were seeded for cloning (300/60-mm dish). For each experimental point, 24 dishes were irradiated with a given exposure at a given wavelength. In each experiment, three or four exposures were used at a test wavelength, and 6 J/m^2 was used at 254 nm (reference cells). After irradiation, 8 ml of culture medium was added and cells were incubated for an additional 6 days to allow for colony growth. The colonies were fixed with methanol and stained with Giemsa. In nonirradiated sets of dishes, the average number of colonies per dish ranged between 60 and 90 in different experiments. Colony appearance was examined with a stereoscopic microscope at ×10 to ×50. Nontransformed colonies displayed a regularly oriented arrangement of cells; the transformed ones exhibited a random criss-cross piling up of cells not seen in the controls. The transformation frequency was calculated on the basis of surviving colonies. Because of the longer irradiation times required for 313 nm light (see Table 1 for exposure rate

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Abbreviations: HEC, Syrian hamster embryo cells; P_i/NaCl, 0.8% NaCl, 0.115% Na_2HPO_4, and KH_2PO_4 and KCl at 0.02% each.

and total exposure), cells were irradiated in one dish and, immediately after irradiation, trypsinized, seeded for cloning (300 per dish), and incubated for 7 days.

Pyrimidine Dimer Assay. For the pyrimidine dimer assay, 5×10^4 cells were plated per 60-mm dish at 24 hr before irradiation. The cells were labeled with [^{14}C]thymidine (0.1 μCi/ml; 50 mCi/mmol; 1 Ci = 3.7×10^{10} becquerels) overnight. The medium was replaced with nonradioactive culture medium at least 1 hr prior to irradiation. After irradiation, the phosphate-buffered saline P_i/NaCl was removed and the dishes were frozen until subjected to DNA extraction. The frozen cells were thawed and lysed with Sarkosyl. After protein was digested with proteinase K, the DNA was purified by phenol extraction. The number of pyrimidine dimers per dalton of DNA was measured by determining the number of incisions resulting from treatment with *Micrococcus luteus* pyrimidine dimer-specific endonuclease as measured by sedimentation in alkaline sucrose. Specific details have been presented (16).

Monochromatic Light Source. The irradiation facility has been described in detail (17). Monochromatic light was provided by a 2.5-kW high-pressure mercury-xenon Hanovia lamp coupled in tandem to two Schoeffel GM 250 single-grating monochromators. An exit slit of 5 mm gave full-width band passes at half-power averaging 5–6 nm for wavelengths from 240 to 313 nm.

Exposure rates were measured by using a UDT 40A optometer (United Detector Technology, Santa Monica, CA). The spectral output of the irradiation facility was initially determined by using the UDT 40A optometer and a spectroradiometer (Cinta double-grating monochromator and associated detector). The spectroradiometer had been calibrated against the output of a FEL type quartz halogen 1000-W secondary standard lamp referenced to a National Bureau of Standards calibrated standard lamp. The optometer was used to monitor the exposure facility output before and after each exposure. The accumulated uncertainty (arithmetical sum of the random and systematic errors and errors due to instabilities in monitoring instruments and high-intensity lamp) in the irradiance measurements was ±21%.

Irradiation Conditions. The medium was removed from the Petri dishes, and the cells were washed once with P_i/NaCl and covered with 2 ml of P_i/NaCl. Uncovered dishes were irradiated individually at room temperature. To prevent possible toxic and mutagenic effects of fluorescent lights on mammalian cells (18) and to reduce possible photoreactivation, the cells were not exposed to any light below 530 nm until they were

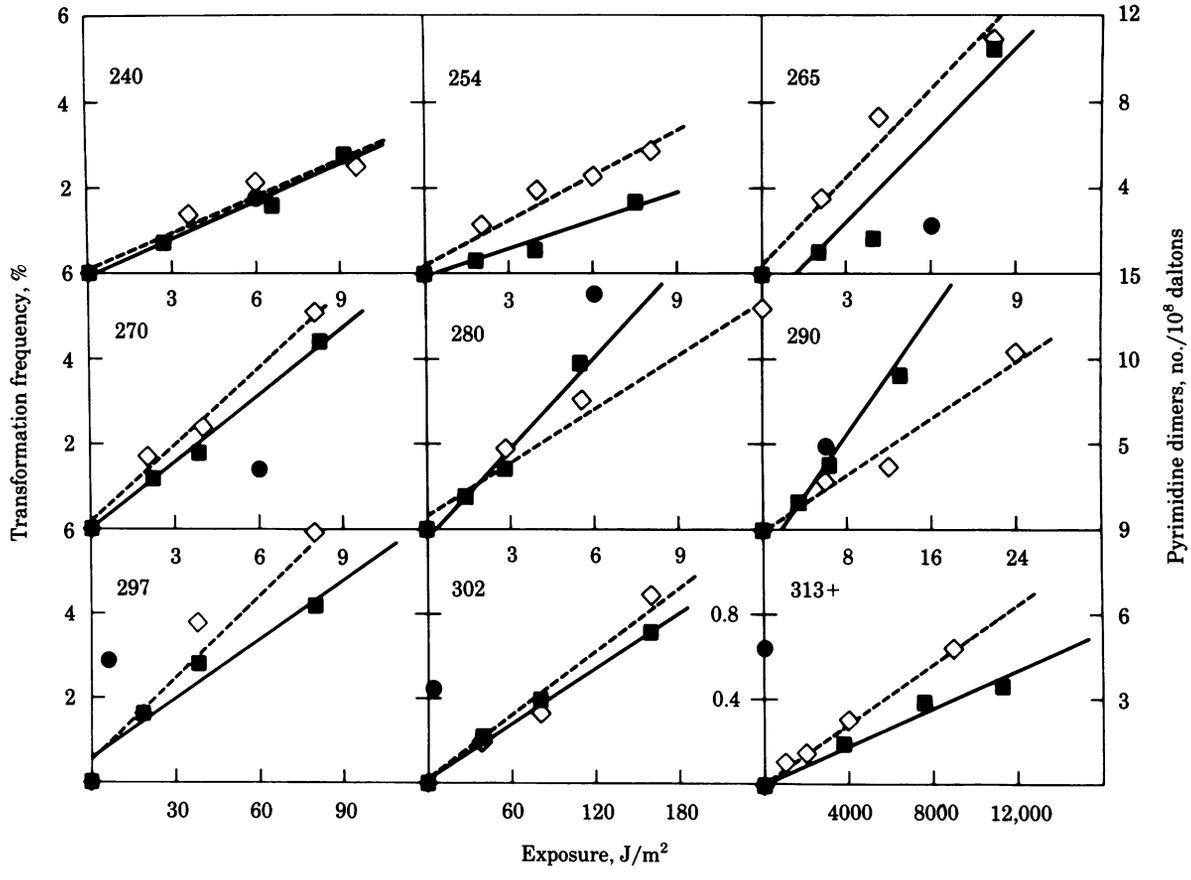

FIG. 1. Wavelength dependence of the induction of transformation and pyrimidine dimer formation in UV-irradiated HEC. The wavelength in nm is given in the upper left corner in each box. +, Mylar filtration. In each transformation experiment, cells were irradiated with various exposures at one wavelength (■) and reference cells were irradiated with 6 J/m^2 at 254 nm (●). In the 313-nm experiment, both the experimental and reference cells were irradiated in single dishes before seeding for cloning. The solid and dashed lines are the linear regression lines for the transformation and pyrimidine dimer data, respectively. The transformation frequency is percentage of colonies with a transformed appearance. The scales for the transformation frequencies at all wavelengths except 313+nm are the same and are shown at the left side of each row. The scale for the transformation frequency at 313+nm is shown at the left of that box. Pyrimidine dimers formation was determined with [^{14}C]thymidine-labeled cells. DNA was extracted and purified after irradiation and treated with pyrimidine dimer-specific endonuclease. The number of pyrimidine dimers per 10^8 daltons of DNA (◇) is equal to 10^8 divided by the number average molecular weight as determined in alkaline sucrose gradients (16). The scales for pyrimidine dimer formation shown at the right side of a row are specific for all boxes in that row.

either fixed and stained or the DNA was extracted. Photoreactivation of pyrimidine dimers by fluorescent lights was not observed in HEC (data not shown).

Experiments with 313-nm light were performed with and without filtration of the light through 0.5-mil-thick (12.7 μm) Mylar to determine the contribution of scattered light emitted by the monochromator. (Mylar transmits 50% of the 313-nm light and <0.5% of wavelengths <302 nm.) The amount of (J/m^2) filtered 313-nm light required was 1.5 to 3 times the amount of unfiltered light required to induce the same level of pyrimidine dimers, lethality, and transformation. Because some of the effect of unfiltered 313-nm light is due to a small amount of scattered, shorter wavelength light, Mylar-filtered 313-nm light was used for the action spectra.

RESULTS AND DISCUSSION

A linear exposure–response relationship for the percentage of transformed colonies and pyrimidine dimer formation in HEC was observed at all wavelengths (Fig. 1). The sensitivity of the cells to transformation and pyrimidine dimer formation was lower at wavelengths 290–313 nm than in the range 240–280 nm. The ratio of the transformation frequency to pyrimidine dimer yield was similar at each wavelength. The data at 313 nm were from cells irradiated in a single dish with Mylar-filtered light. When HEC were treated with a carcinogen (UV, benzo[*a*]pyrene, etc.) in a single dish before being seeded for cloning, the transformation frequency was 2–3 times lower than when cells were seeded for cloning before treatment. This accounts for the low transformation frequency shown for the 313-nm experiment. The shape of the exposure response curves for lethality was similar at each wavelength tested. Typical survival curves are shown in Fig. 2. The sensitivity of the cells for lethality was also wavelength dependent and lower at the longer wavelengths. The shape of the survival curves was also similar to those found in other action spectra studies utilizing mammalian cells (19, 20).

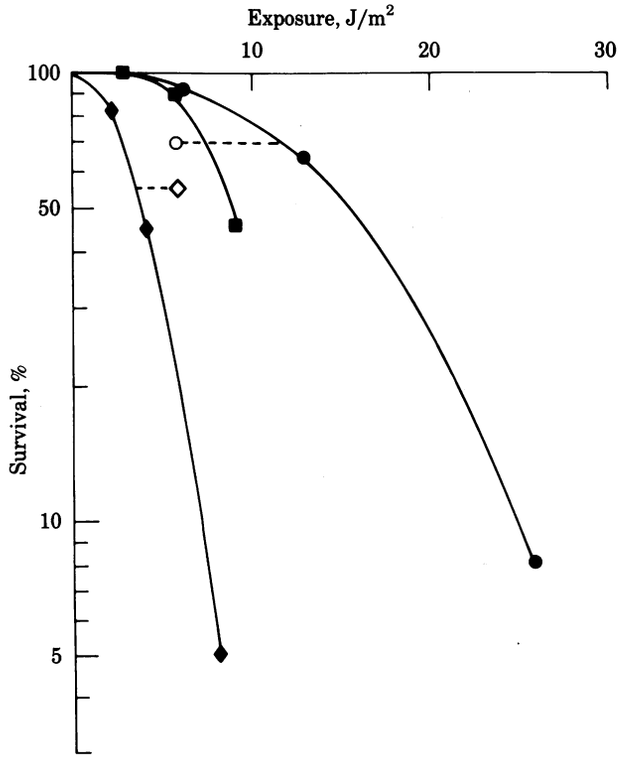

FIG. 2. UV-induced lethality of HEC at three wavelengths (■, 254 nm; ◆, 270 nm; ●, 290 nm). Nonirradiated controls had 60–90 colonies per dish. Percentage survival is the cloning efficiency of the irradiated cells divided by that of nonirradiated cells multiplied by 100. Reference cells were irradiated with 6 J/m^2 at 254 nm each time a given experiment was performed. The survival of the reference cells is shown by the open symbols. The dashed lines indicate the equivalent survival. The equivalent exposure for lethality is the dose that gives the equivalent survival.

Table 1. Exposures required for specific effects at different wavelengths of UV light

Wavelength, nm	Exposure rate, W/m^2	Exposure for effect, J/m^2			
		Pyrimidine dimer*	Transformation†	Lethality‡	Mutagenesis§
240	1.1	1.7	5.8¶	9.3¶	NT
254	0.34	0.95	6.0	6.0	23
265	0.38	0.73	4.3¶	4.0¶	6.3
270	0.51	0.64	2.6	3.5	4.0
280	1.3	1.0	8.6¶	5.6¶	6.0
290	1.3	2.3	7.4	12	NT
297	3.8	8.7	47	75	47
302	3.2	25¶	97¶	180¶	190
313+‖	5.7	1800¶	12,000¶	12,000¶	NT

* The exposure that induced the formation of 1 pyrimidine dimer per 10^8 daltons of DNA.
† The exposure that induced the equivalent frequency of transformation as 6 J/m^2 of 254-nm light.
‡ The exposure that induced the equivalent lethality as 6 J/m^2 of 254-nm light.
§ The exposure that induced 4×10^{-4} BrdUrd-resistant mutants per survivor in mouse lymphoma cells. This mutation frequency is 10-fold higher than the spontaneous mutation frequency at this locus. The exposure was extrapolated from curves in which the mutation frequency was plotted versus exposure. NT, not tested.
¶ The mean of two or three experiments.
‖ Irradiation filtered by Mylar.

Both the transformation frequency and the lethality induced in HEC by 6 J/m^2 of 254-nm light from the monochromatic source varied from experiment to experiment (Fig. 1, solid circles; Fig. 2, open symbols). To control for this variability, reference sets of cells were irradiated with 6 J/m^2 of 254-nm light in each experiment. The linear regression line of the exposure–response curves for transformation induced at a given wavelength was calculated in each experiment (Fig. 1, solid line) and the exposure that induced a transformation frequency equivalent to that induced by 6 J/m^2 of 254-nm light (Fig. 1, closed circle) was determined. The exposure-equivalent for lethality was extrapolated by comparing the data obtained with the reference cells (6 J/m^2 at 254 nm) with those at the other wavelength (Fig. 2). When a series of experiments was performed at a given wavelength, the exposure-equivalent for either transformation or lethality differed by no more than 20% of the mean and usually was within 10%. Linear regression analysis of pyrimidine dimer production (Fig. 1) was used to determine the energy (in J/m^2) required to form 1 pyrimidine dimer per 10^8 daltons of DNA at each wavelength.

The exposure-equivalents for transformation and lethality and the exposure found to yield 1 pyrimidine dimer per 10^8 daltons of DNA at each wavelength are compared in Table 1. The most effective wavelength for transformation and lethality was 270 nm because at this wavelength the lowest exposure was required to give the equivalent effect. Furthermore, the lowest exposure required to form 1 pyrimidine dimer per 10^8 daltons was also at 270 nm. For comparative purposes, the exposure required to induce 4×10^{-4} BrdUrd-resistant mutants per survivor in mouse lymphoma cells is included (17); again, the most effective wavelength was 270 nm.

Because the shapes of the exposure–response curves were the same at all wavelengths, the criteria for establishing an action spectrum have been fulfilled (21). The exposures shown in Table 1 were converted from J/m^2 to quanta/m^2. For each end point, the relative sensitivity per quantum at each wavelength was normalized by dividing the exposure (quanta/m^2) for 254 nm by that at the given wavelength. The action spectrum (Fig. 3) is the relative sensitivity per quantum plotted versus wavelength. The action spectrum for pyrimidine dimer formation is biologically significant because the cells were irradiated with UV in the same exposure range as were the cells used for the other two assays. The action spectra for transformation, lethality, and pyrimidine dimer formation were essentially the same; the most effective wavelengths in all three cases are in the range 265–270 nm.

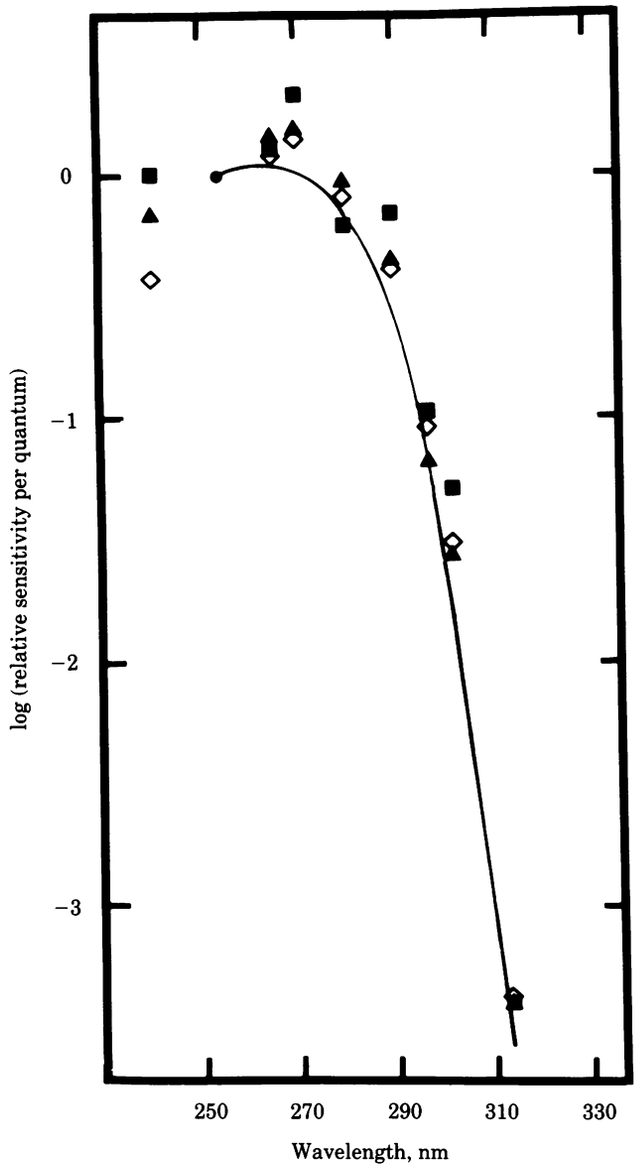

FIG. 3. Action spectra for transformation (■), lethality (▲), and pyrimidine dimer formation (◇) in HEC. Results at other wavelengths were normalized to those at 254 nm (designated as 1.0). The data for 313 nm were from the experiments in which Mylar filtration was used. The solid line represents an average action spectrum for lethality, mutagenesis, bacteriophage survival, and pyrimidine dimer production as measured in bacterial systems; data from Setlow (6) were normalized to 254 nm.

Action spectra similar to ours have been observed for lethality and pyrimidine dimer production in Chinese hamster V79 cells (19), killing of nondividing normal and xeroderma pigmentosum human cells (20), lethality and mutagenesis in L5178Y mouse lymphoma cells (17), and induction of chromosome aberrations in Chinese hamster ovary cells (22). The action spectrum for transformation (Fig. 3) is also similar to the average action spectrum measured in bacterial systems for various effects such as lethality, mutagenesis, bacteriophage survival, and pyrimidine dimer production (solid line in Fig. 3). This average bacterial action spectrum was used by Setlow (6) in the development of a theoretical analysis of the wavelengths in sunlight effective in producing skin cancer. The assumption that the average bacterial action spectrum is a good approximation of the effectiveness of wavelengths for carcinogenesis was correct because of the similarity between the action spectra for mammalian cell transformation and of diverse effects on bacteria. The theoretical analysis of the wavelengths relevant to sunlight-induced cancer can now be further developed.

Because the DNA absorption maximum is at 258–260 nm, one might expect that this range of wavelengths would be most effective in producing pyrimidine dimers. However, as shown in Fig. 3, the most effective wavelength range for pyrimidine dimer induction in UV-irradiated HEC was 265–270 nm. This is consistent with the maximum absorption of UV light by pyrimidines in the range 265–270 nm. The absorption of light by pyrimidines alone may lead to dimer formation with little contribution by energy transferred from excited purines. The maxima in other action spectra for lethality or pyrimidine dimer production are within 260–280 nm (6, 8–13, 17, 19, 20, 22–24). The variation in maxima probably reflects small differences in the relative sensitivity per quantum in this range. Although our data suggest a pyrimidine chromophore, DNA is not excluded.

The similarity among the action spectra for pyrimidine dimer formation, lethality, and induction of transformation of HEC suggests that there is one target for all these effects. UV, x-irradiation, and most chemicals that are carcinogens or mutagens damage DNA. As a consequence, the mutational model of carcinogenesis has received wide support. The most convincing evidence that implicates DNA damage in carcinogenesis comes from studies of sunlight-induced human skin cancer and of UV-induced lesions in the Amazon molly (*Poecilia formosa*). Humans with the genetic disease xeoderma pigmentosum are highly sensitive to UV-induced skin cancer. Cells from these individuals are defective in one or more mechanisms for repairing DNA damage (25). Results with Amazon mollies indicate an association between pyrimidine dimers and a step in carcinogenesis. When UV-irradiated cells were injected into these fish, lesions in the thyroid resulted. However, when the cells were illuminated with photoreactivating light before injection, in order to remove pyrimidine dimers but not other photoproducts, the incidence of these lesions was reduced to 1/10th (26).

DNA is the target for pyrimidine dimer production. The evidence that the action spectra for transformation and cellular pyrimidine dimer production are similar supports the concept that DNA is also the target for UV-induced carcinogenesis.

Dosimetry of the monochromatic light source was kindly performed by O. Ellingson, R. G. Bostrom, and C. W. Hench II, of the Division of Electronic Products, Bureau of Radiological Health. Excellent technical assistance was provided by Mr. Anthony DeMarinis. Dr. R. B. Setlow provided the data used for constructing the average bacterial action spectrum.

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