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Synergistic Effects of Wavelengths of Ultraviolet Radiation on Capacity in a Mammalian Cell-Virus System In Vitro

Bobby Cobb

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SYNERGISTIC EFFECTS OF WAVELENGTHS OF ULTRAVIOLET RADIATION ON CAPACITY IN A MAMMALIAN CELL-VIRUS SYSTEM IN VITRO

A Thesis
Presented to
the Faculty of the Department of Biology

Western Kentucky University
Bowling Green, Kentucky

In Partial Fulfillment
of the Requirements for the Degree
Master of Science

by
Bobby E. Cobb
August, 1978
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SYNERGISTIC EFFECTS OF WAVELENGTHS OF ULTRAVIOLET RADIATION ON CAPACITY IN A MAMMALIAN CELL-VIRUS SYSTEM IN VITRO

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SYNERGISTIC EFFECTS OF WAVELENGTHS OF ULTRAVIOLET RADIATION ON CAPACITY IN A MAMMALIAN CELL-VIRUS SYSTEM IN VITRO

Bobby E. Cobb
August, 1978

Directed by: Dr. Thomas P. Coohill, Dr. Fernando Morgado, and Dr. T. Alan Yungbluth

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The synergistic effect between each of two minor contaminating UV wavelengths has been studied. The TC-7 host cell-capacity system was used. The macromolecular damage induced in monolayers of cell cultures as the result of exposure to UV radiation was measured by determining the ability of irradiated cells to support the replication of Herpes simplex virus. The macromolecule involved has been shown to be DNA and the damage is probably due to the formation of thymine dimers plus some undefined chromophore associated with the DNA of the cell.

Preliminary experiments showed that low exposures of germicidal radiation are less effective in decreasing the survival of capacity while higher exposures are more effective when compared to monochromatic 254 nm radiation. Subsequent experiments showed a synergistic interaction between 254 nm and either 295 nm or 313 nm radiation at doses comparable to those emitted from a germicidal lamp as well as greatly increased doses.
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INTRODUCTION AND LITERATURE REVIEW

Ultraviolet (UV) radiation has become an effective tool in studies on biological materials, particularly at the subcellular level. Early work with UV radiation has produced such effects as cell death (Zelle and Hollaender, 1955), mutation (Zetterberg, 1964) and latent virus activation (Franklin, 1954) in bacterial cells. More recent work has shown similar results in mammalian cells (Kaplan, et al., 1975; Todd, et al., 1968). UV radiation studies were among the first to show that nucleic acids are the biomolecules involved in reproduction and mutation (Gates, 1930).

The majority of studies performed with UV utilize wavelengths in the so-called far-UV (200 nm - 300 nm) and near-UV (300 nm - 400 nm) regions. Molecules with conjugated double bonds (alternating single and double carbon bonds) are strong far-UV absorbers. Since all nucleic acid residues are heterocyclic aromatic compounds, they are therefore effective far-UV absorbers, the pyrimidines being more sensitive than the purines. Peak absorption for nucleic acids is at 260 nm while proteins peak at 280 nm. However, nucleic acids absorb 10 - 20 times as much UV as protein in the 240 nm - 290 nm region because of the number of conjugated bonds.

Many major advances in photobiology have been made using monochromatic radiation, especially those involving the identification of chromophores (light-absorbing biological
molecules). However, recent experiments demonstrate that using single monochromatic wavelengths may fail to illustrate the total basis of the biological effects observed. Peak et al. (1975) have found a synergistic effect between near-UV wavelengths (365 nm and either 313 nm or 334 nm) in the inactivation of transforming DNA. They showed that DNA is twofold (313 nm) or threefold (334 nm) more sensitive to 365 nm radiation when one of the minor wavelengths is added than to 365 nm radiation alone. When either of the minor wavelengths was used alone the same exposures were insufficient to elicit a significant decrease in DNA activity.

Synergism may be defined here as an interaction between two or more wavelengths of radiation that produce an effect greater than the sum of the individual radiation treatments administered independently. Mackay et al. (1976) have proposed a synergistic interaction between far- and near-UV in the inactivation of Salmonella typhimurium and Escherichia coli. Other investigators have shown similar effects using pre-UV treatment and gamma radiation (Lewis, et al., 1975) and UV plus mild sub-lethal heat (Tyrrell, 1976).

Coohill et al. (1977) used capacity as an indicator of the amount of cellular damage induced in mammalian cells by UV radiation. Capacity is defined as the ability of a cell to support the growth of a particular virus (Benzer and Jacob, 1953). UV-irradiation of the cell usually decreases its ability to support viral growth (Anderson, 1948; Bockstahler
and Lytle, 1970). This is because DNA transcription must occur in order for viral replication to take place. It has been shown that the UV action spectrum for inactivation of host capacity closely follows the absorption spectrum for nucleic acid. DNA is implicated as the major target molecule for the phenomenon of capacity (Lytle and Benane, 1975; Coppey and Nocentini, 1976; Coohill, et al., 1977). At least part of the mechanism involved is believed to be thymine dimer formation.

The presence of a thymine dimer in a cell's DNA interrupts the sequence of the DNA bases and halts the transcription of mRNA. This ultimately blocks the production of the enzyme or protein produced by the gene in which a dimer occurs (Chu, 1965; Trosko, et al., 1965). If the dimers are not repaired, cellular death follows which is reflected in the surviving fraction of the particular cells under study.

Fortunately, many cells possess mechanisms to repair damage from thymine dimer formation. Photoreactivation, a light-triggered enzymatic reaction, is found in all cells except a few bacterial and placental mammalian systems (Jagger, 1958; Rupert, 1964). Another such mechanism is dark, or excision, repair (Lehninger, 1975).

In the course of the study on capacity by Coohill et al. (1977) the UV wavelengths were produced by a monochromatic light source. They found 254 nm to be one of the most effective wavelengths in inducing thymine dimer formation (Lehninger, 1975). However, similar experiments performed in other
laboratories have had as the source of UV radiation a fluorescent-type germicidal lamp. The germicidal lamp emits 86% of its total energy output at 254 nm and 14% at minor contaminating wavelengths.

Researchers in Coohill's laboratory noticed a difference in the decrease of capacity when monochromatic 254 nm radiation and germicidal radiation survival curves were compared (T. P. Coohill, Personal Communication). This finding was confirmed and elucidated further by the author of this text. Data show that monochromatic 254 nm radiation is more effective in reducing capacity at low exposures while germicidal radiation is more effective at higher exposures.

The purpose of the present work is to determine if a synergistic interaction occurs between the primary 254 nm radiation and the minor contaminating wavelengths inherent in the output of germicidal lamps when used in the irradiation of a mammalian cell-virus system.
MATERIALS AND METHODS

Cell cultures

An established cloned line of African green monkey kidney epithelial cells (TC-7) was obtained from Dr. G. Zamansky of the Massachusetts General Hospital, Boston, MA. This cell line was originally established as a clone of the CV-1 cell line by J. A. Robb (Robb and Huebner, 1973). These cells were maintained in a growth medium consisting of 1X Dulbecco's Minimum Essential Medium (DMEM) with the following components added per liter (unless otherwise noted all components were obtained from Grand Island Biological Company, Grand Island, NY) when prepared: 2 g 8-D-glucose, 0.3 g arginine HCl, 0.02 g histidine HCl, 1.5 g L-glutamine, 15 mM each HEPES, TES, and MOPS, and 2 g sodium bicarbonate. The medium was buffered to a pH of 6.8 - 7.0 with 10 N NaOH and filter sterilized through a 0.22 μm-pore membrane filter. The medium was sterility tested at 37°C for at least three days before use. Ten per cent fetal bovine serum, 16 ml 50X amino acids, 8 ml 100X vitamins, 100 units/ml penicillin, 100 μg/ml streptomycin and 0.25 μg/ml fungizone were added prior to use of medium in culture.

Stock cultures were grown in 150 cm² closed flasks (Corning Plastics Co., Corning, NY) at 37°C. Prior to experimental use cells were removed from stock cultures by trypsinization with a solution of 0.025 % each of trypsin 1:250 EGTA dissolved in Mg²⁺- and Ca²⁺-free phosphate-buffered
saline (PBS) at pH 7.8 and transferred to 60 mm² petri dishes
(Falcon Plastics Co., Oxnard, CA) in 4 ml of growth medium
per dish. These cells were incubated and allowed to grow
until freshly confluent monolayers were obtained.

**Viral assay: Plaque-forming ability (pfa)**

A macro-plaque forming strain of HSV-1 (Herpesvirus
hominus Type 1) was obtained from Dr. C. D. Lytle of the
Bureau of Radiological Health, Rockville, MD. These viruses
were inoculated into confluent monolayers of TC-7 cells and
incubated until cytopathic effects were evident, then
harvested by centrifugal separation and stored in vials at
-40°C until used in experimental viral assay.

For viral assay, freshly confluent monolayers of cells
were inoculated with an appropriate viral titer in 2 ml of a
maintenance medium consisting of 1X DMEM growth medium
supplemented with 2% fetal bovine serum and antibiotics as
described above. Cells were then incubated at 37°C for 90
min to allow viral adsorption.

The viral inoculum was removed and 4 ml of growth medium
containing 0.25% immune serum globulin (ISG) (Armour
Pharmaceutical, Kankakee, IL) was added to prevent non-cellular
viral transfer and to refeed the cells. The cells were then
incubated for 48 hrs at 37°C to allow viral growth and cell-
to-cell transfer.

The monolayers were stained by removing the medium plus ISG
and adding 2 ml of a 3% aqueous solution of crystal violet
in 20% ETOH and 0.8% ammonium oxalate (Carolina Biological
Co., Burlington, NC). The solution was removed after 10 min
and plaques counted.

Viral dilutions were adjusted so that approximately 100
plaque-forming units (pfu's) were inoculated onto unirradiated
control dishes and 250 pfu's onto irradiated monolayers.

**Germicidal exposures**

Confluent monolayers were irradiated in open petri dishes
with a 15 W germicidal lamp (G15T8, General Electric Co.,
Schenectady, NY) with 86 % of its output at 254 nm and 14 %
at other contaminating wavelengths. The lamp was mounted in
a wooden box equipped with a shutter to control exposure of
the cells to the germicidal radiation (Figure 1). Open petri
dishes were placed 41 cm from the UV radiation source.

Photocurrent from the UV source was measured using a J-225
Short Wave Sensor Cell (U-V Products, Inc., San Gabriel, CA)
and a volt-ohm-microammeter (Simpson Electric Co., Chicago,
IL). The value of the photocurrent output was converted to
energy exposure rate. Exposure rates were measured at the
beginning and end of each experiment. Total exposure was
equal to the length of time the shutter was open multiplied
by the exposure rate.

**Monochromatic exposures**

The source of 254 nm monochromatic radiation was a 2.5 kW
high pressure mercury-xenon compact arc lamp (929B Hanovia
lamp, Canrad-Hanovia, Newark, NJ). This source was air-cooled
Figure 1. Apparatus used for germicidal lamp exposures.
RAD\textsuperscript{I}A\textsuperscript{T}\textsuperscript{I}ON SOUR\textsuperscript{C}E

\textbf{SOURCE 254 nm}

\textbf{SHUTTER}

\textbf{UV}

\textbf{CELLS}
to remove ozone while infrared radiation was partially removed by means of a circulating water filter.

Spectral separation was achieved by means of two GM 250 25 cm diffraction-grating monochromators coupled in tandem (Schoeffel Instruments, Westwood, NJ) (Figure 2). Half-band width was 6.0 nm with a slit width of 4.0 mm. Spectral output was found to be as stated within the limits of our testing.

Exposure rates were measured at the beginning and the end of each experiment by placing a calibrated UV-sensitive photodiode (Cal-UV, United Detector Technology, Inc., Santa Monica, CA) in the sample position and measuring the photocurrent produced with a Keithley 610B Electrometer (Keithley Instruments, Cleveland, OH). Photocurrent was again converted to energy exposure rate.

Addition of a second monochromatic wavelength to the primary 254 nm wavelength was accomplished by means of a 200 W mercury lamp (HBO 200 W Super Pressure Mercury Lamp, Osram Corp., Berlin, Germany) enclosed in a structural addition to the diffraction-grating monochromator (Figure 3). This structure was designed and built by the author for this specific purpose. This UV source was also air-cooled to remove ozone and heat. A 645L Fluorolume power supply (American Optical Co., Buffalo, NY) was used to ignite and power the lamp. Spectral separation of this source was achieved by means of Ealing-TFP interference filters (Ealing Corp., Cambridge, MA) with half-band widths of 15 nm and 12 nm present in the 295 nm and 313 nm filters, respectively. The filters were positioned in a holder between
Figure 2. Two GM 250 diffraction-grating monochromators coupled in tandem used for monochromatic exposures.

(From Coohill, et al. (1977) with permission of the authors.)
Figure 3. Structural addition to the diffraction-grating monochromator housing a 200 W mercury lamp for additional monochromatic wavelengths.
a front-surfaced aluminized mirror and the sample dish.

The radiation beams from both sources were directed downward by means of two front-surfaced aluminized mirrors. These mirrors were oriented so that the beams from both sources would be coincident on the sample dish during exposures. The 200 W source was adjustable in three dimensions to allow for refinement of beam alignment. An adjustable diaphragm between the 200 W source and its front-surfaced mirror allowed variation of exposure rates and beam size. Shielding was provided to prevent stray contaminating light. Exposure rates were measured in the same manner as for the grating monochromator.

Cells were irradiated in horizontally-oriented open petri dishes. Dishes were rotated at 0.5 rps to compensate for possible inhomogeneities in the radiation beams due to currents in the circulating water filter or to aberrations in the quartz envelopes of the lamps. Reciprocity of time and intensity of the beams was tested and no dependence of capacity on intensity was observed.

Before exposure to any radiation the growth medium was removed from confluent monolayers. The monolayers were rinsed twice in 2 ml of Dulbecco's PBS (Dulbecco and Vogt, 1954) to remove UV-absorbing components in the medium which could form toxic photoproducts. A final 2 ml of PBS was placed on the monolayers to keep them moist during irradiation. Immediately after irradiation the PBS was removed and the cells inoculated with unirradiated viruses in maintenance medium.
RESULTS

Survival curves were obtained by plotting the percent survival of plaque-forming virions (a measure of capacity) on the ordinate and exposure on the abscissa of a two-cycle semi-logarithmic graph. The number of plaques on control dishes was considered 100% survival and normalized to 1.0. The number of plaques on experimental (irradiated) dishes was calculated as a percentage of control values. The amount of UV radiation from the 254 nm source to which experimental dishes were exposed is shown as mJ/cm². Values from individual experiments were normalized, then averaged, and the standard deviation determined for all experimental points and shown as error bars.

Figure 4 shows the results of eight preliminary experiments performed to determine if there is a difference between the effectiveness of monochromatic and germicidal 254 nm radiation in reducing capacity. There is a difference between the two radiation sources at almost all exposures tested. A Student's 't' test was used to determine statistical differences between the two sources at each exposure. The results of these tests are shown in Table 1.

An interesting property of these two survival curves is that they cross in the region between 4.0 and 5.0 mJ/cm². At lower exposures the monochromatic 254 nm radiation is more effective in reducing capacity while at higher exposures the
Figure 4. A comparison of the survival curves of germicidal and monochromatic 254 nm radiation with error bars showing standard deviations.

Germicidal 254 nm - ○
Monochromatic 254 nm - △
TABLE 1

A Statistical Analysis of the Comparison of the Survival Curves of Germicidal Radiation and 254 nm Monochromatic Radiation

<table>
<thead>
<tr>
<th>Exposure (mJ/cm²)</th>
<th>Monochromatic Avg.</th>
<th>Monochromatic s.d.</th>
<th>Germicidal Avg.</th>
<th>Germicidal s.d.</th>
<th>Σ</th>
<th>t</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>--</td>
<td>100</td>
<td>--</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>83</td>
<td>±6</td>
<td>97</td>
<td>±5</td>
<td>5.52</td>
<td>2.53 *</td>
</tr>
<tr>
<td>1.0</td>
<td>77</td>
<td>±9</td>
<td>93</td>
<td>±5</td>
<td>7.28</td>
<td>2.19 *</td>
</tr>
<tr>
<td>2.0</td>
<td>59</td>
<td>±8</td>
<td>77</td>
<td>±8</td>
<td>8.00</td>
<td>2.25 *</td>
</tr>
<tr>
<td>3.0</td>
<td>42</td>
<td>±8</td>
<td>47</td>
<td>±11</td>
<td>9.60</td>
<td>0.52</td>
</tr>
<tr>
<td>4.0</td>
<td>24</td>
<td>±5</td>
<td>35</td>
<td>±7</td>
<td>6.08</td>
<td>1.89</td>
</tr>
<tr>
<td>5.0</td>
<td>8</td>
<td>±2</td>
<td>4</td>
<td>±1</td>
<td>1.58</td>
<td>-2.53 *</td>
</tr>
<tr>
<td>6.0</td>
<td>3.3</td>
<td>±0.7</td>
<td>1.5</td>
<td>±0.7</td>
<td>0.65</td>
<td>-2.77 *</td>
</tr>
</tbody>
</table>

\[ t = 0.05 = 2.145 * \]
\[ 0.01 = 2.977 ** \]
germicidal lamp is more effective. One possible explanation
for this phenomenon may be that at lower exposures the minor
wavelengths cancel part of the damage caused by the 254 nm
radiation. At higher exposures more energy is available
from the minor wavelengths than is available at lower
exposures. This extra energy may be sufficient to play a
substantial role in inducing cell DNA damage, therefore
reducing capacity. This increased damage may be due directly
to the cell DNA (although thymine dimer formation by the
minor wavelengths is extremely unlikely), or to some
molecule associated with the DNA which can transfer the added
energy to the DNA and in some way induce damage.

In the subsequent synergism experiments the exposures of
254 nm radiation given were between 3.8 mJ/cm² and 4.8 mJ/cm².
Exposures in this region reduced capacity to approximately a
10 % survival level (F₁₀).

The two minor contaminating wavelengths emitted from
germicidal lamps tested for their synergistic potential were
295 nm and 313 nm. In these experiments survival of capacity
is again plotted against exposure. Fifteen experiments were
performed with each wavelength. The data from all experiments
at each minor wavelength were normalized to the lowest
exposure before statistical analysis. Since the data were
normalized to the lowest exposures there was no standard
deviation at these points. At all other points the standard
deviations are shown as error bars.

For each of the two minor wavelengths the percentage of
radiation emitted by a germicidal lamp at that particular wavelength was determined (Table 2). The energy emitted by each of these wavelengths in a total exposure of 4.3 mJ/cm² was then calculated and used as the amount of energy added to the monochromatic 254 nm as the dose inherent in the germicidal lamp (0.02 mJ/cm² for 295 nm and 0.1 mJ/cm² for 313 nm). The value of 4.3 mJ/cm² for which the minor doses were calculated was chosen because it is the average of the exposures delivered to the irradiated dishes at 254 nm. The use of a minor dose comparable to each individual 254 nm exposure was not feasible since the difference in low dose exposures would be only fractions of a second and could not be achieved on the equipment available. Therefore an average value was used to standardize procedures. These minor wavelength exposures were administered during the 254 nm exposures although they were substantially shorter in duration.

A higher dose of each of the minor wavelengths was also added to the 254 nm radiation. This higher dose was 200 times the normal (low) dose for 295 nm (2.0 mJ/cm²) and 10 times the normal dose for 313 nm (1.0 mJ/cm²). The rationale for these orders of magnitude was that they allowed exposure times which could be administered simultaneously with the 254 nm exposures without being substantially longer.

The result of the addition of the normal dose of 295 nm to the 254 nm radiation (Figure 5) shows evidence of a synergistic effect in reducing capacity. Theoretical calculations
Figure 5. The survival curves of 254 nm radiation plus added normal (germicidal) and high doses of 295 nm radiation.

254 nm - O

254 nm plus normal 295 nm - △

254 nm plus high 295 nm - □

Theoretical 254 nm plus high 295 nm - - -
of the simple addition of the higher dose of 295 nm to the monochromatic 254 nm were made and are shown along with the survival curves as a dashed line. There is a greater reduction in capacity with the addition of the normal 295 nm dose than would be expected from the theoretical addition of the higher dose. The experimental addition of the higher 295 nm dose is much more effective in reducing capacity than either the normal dose or the theoretical higher dose. Therefore, there seems to be a synergistic interaction between 295 nm and 254 nm radiation.

The addition of 313 nm to the monochromatic 254 nm gives similar but even more striking results (Figure 6). Since 313 nm radiation alone has no effect on reducing capacity, even at extremely high exposures (Figure 7) (seemingly causing no overt damage to cell DNA), the theoretical dashed line for the addition of the higher dose of 313 nm does not vary from the line for 254 nm alone. Therefore, any decrease in capacity when 313 nm radiation is added would indicate a synergistic effect.

This is shown for the addition of both the normal and higher 313 nm doses. With the exception of the 4.8 mJ/cm² 254 nm plus normal 313 nm exposure point, both the 313 nm survival curves show greater decreases in capacity than those for the addition of 295 nm to 254 nm radiation. This would indicate an even greater synergistic interaction between 313 nm and 254 nm than between 295 nm and 254 nm. This could be explained for the normal dose survival curves since the exposure
Figure 6. The survival curves of 254 nm radiation plus added normal (germicidal) and high doses of 313 nm radiation.

254 nm - ○
254 nm plus normal 313 nm - △
254 nm plus high 313 nm - □

Theoretical 254 nm plus high 313 nm - ---
of 313 nm is greater than that of 295 nm, even though 295 nm radiation is inherently more energetic than 313 nm radiation (295 nm photons possess more energy than 313 nm photons).

However, when the two higher dose survival curves are compared, the 295 nm radiation is less effective in reducing capacity. From this, it seems that the chromophore which is absorbing the light energy is more sensitive to 313 nm radiation than to 295 nm radiation.

The possibility of contaminating stray radiation of a damaging wavelength from the 200 W source affecting the synergistic interactions was feasible even though shielding was provided. In order to determine if the shielding was adequate the control dishes were handled under three different conditions. One group was sham irradiated with the dishes open for the same period as the longest experimental dish in each experiment. The second group was placed near the irradiation area with dishes open for the same period of time. The third group was not taken into the irradiation area. Plaque counts on the first two groups did not vary significantly from the third group.
DISCUSSION

It seems evident that at least part of the difference between the survival curves of TC-7 cells exposed to germicidal light and monochromatic 254 nm radiation in vitro is due to a synergistic interaction between 254 nm plus 295 nm and 313 nm UV radiation. However, no assumptions can be made at this time concerning the other minor contaminating wavelengths emitted from the germicidal lamps. These other wavelengths may produce a synergistic, antagonistic, or no effect when added to the primary 254 nm wavelength. The elucidation of this problem awaits further investigation.

The seeming disparity seen in the preliminary experiments comparing germicidal and monochromatic 254 nm radiation in which the survival curves cross can be given at least one plausible explanation. If the monochromatic 254 nm survival curve is assumed as a base line, then the germicidal survival curve may be considered as a deviation from that base line. Jagger (1972) has shown a photoprotective effect between 254 nm radiation and low levels of near-UV on DNA. This could account for the increased capacity at low total exposures of the germicidal lamp since low levels of the minor near-UV wavelengths are available. As the total germicidal exposure increases the level of the minor wavelengths also increases. Higher levels of minor near-UV wavelengths have been shown to produce a photosensitization to 254 nm radiation (Tyrrell
and Webb, 1973). This could account for the decrease in capacity at the higher total exposures. The crossover point of the survival curves may be a null region where these two effects cancel each other during the transition from one to the other.

Although there is no definite mechanism proposed for synergism in bacterial and mammalian systems, Peak et al. (1975) have proposed three hypothetical mechanisms for synergism between near-UV wavelengths on transforming DNA. These are summarized as follows: (1) additional radiation may alter the DNA so as to leave genetic activity unchanged, but cause primary wavelength-induced lesions to be inaccessible to repair; (2) minor UV wavelengths induce 'sub-lethal' lesions which make DNA more sensitive to the primary wavelength; or, (3) a sensitizing molecule may bind to the DNA and be altered by a minor wavelength in such a way that absorbance at primary wavelengths is increased.

An even more attractive model would be a variation on a combination of Peak's second and third mechanism. A chromophore may absorb the energy from a photon of a minor wavelength. Like all molecules this one absorbs more strongly those photons with certain discrete energy levels. The extra energy stored in the excited chromophore may be transferred to DNA thus raising the energy level in certain chemical bonds making these bonds more sensitive to the effects of the primary wavelength. Although there is no conclusive evidence for this hypothesis it fits the data presented here.
Whatever the mechanism(s) involved in synergism, there are some practical implications which should be considered. UV radiation is more widely found in both research and applied use today than ever before. Investigators, particularly those using germicidal or other broad-spectrum type lamps, should be made aware of the possible synergistic interactions between UV wavelengths. Attention should be drawn to the fact that observations may occur as a result of the multiple component wavelengths inherent in their source of radiation.

Many workers in the field of UV photobiology have access to both broad-spectrum and pure (monochromatic) radiation sources. They should especially be wary when comparing results of experiments performed using different sources of UV. An effort should be made to re-evaluate published data as well.
Table 2. Spectral Emission Lines of a General Electric G15T8 Germicidal Lamp with Corresponding Percentages of Total Energy Output

Figure 7. A Comparison of the Survival Curves of 254 nm, 295 nm and 313 nm Monochromatic Wavelengths
Table 2

Spectral Emission Lines of a General Electric G15T8 Germicidal Lamp with Corresponding Percentages of Total Energy Output

<table>
<thead>
<tr>
<th>Wavelength (nm)</th>
<th>% Total Output</th>
</tr>
</thead>
<tbody>
<tr>
<td>254</td>
<td>85.65</td>
</tr>
<tr>
<td>295</td>
<td>0.35</td>
</tr>
<tr>
<td>313</td>
<td>1.90</td>
</tr>
<tr>
<td>334</td>
<td>0.10</td>
</tr>
<tr>
<td>365</td>
<td>2.00</td>
</tr>
<tr>
<td>405 - 408</td>
<td>2.00</td>
</tr>
<tr>
<td>436</td>
<td>5.00</td>
</tr>
<tr>
<td>546</td>
<td>2.50</td>
</tr>
<tr>
<td>577 - 590</td>
<td>0.50</td>
</tr>
<tr>
<td><strong>100.00</strong></td>
<td></td>
</tr>
</tbody>
</table>

(with the permission of Mr. Edward T. Ryan, III, Personal Communication)
Figure 7. A comparison of the survival curves of 254 nm, 295 nm and 313 nm monochromatic wavelengths.

- 254 nm - ○
- 295 nm - △
- 313 nm - □
Survival of capacity vs. exposure (mJ/cm²)


