ABSTRACT

The uncontrolled exposure of Fischer’s medium to cool white fluorescent (CWF) light or other sources emitting near-ultraviolet or visible light absorbable by riboflavin is a crucial random variable in experiments which utilize L5178Y cells and this medium. The radiation effects of CWF light result in the rapid development of toxic photoproducts in the medium which are cytostatic at lower doses of radiation and cytotoxic at higher doses. After a 24-hr suspension in medium irradiated for 3 or 48 hr, the cloning efficiencies of cells subsequently plated in light-protected medium were 87 and 3%, respectively. The corresponding near-ultraviolet doses for these periods of exposure to CWF light were 0.22 x 10^4 for a 3-hr exposure and 3.47 x 10^4 J/sq m for a 48-hr exposure. Cells incubated in lightly irradiated medium resumed growth at nearly normal rates following a 24- to 48-hr period in which no increase in cell numbers occurred. Exposure of medium containing riboflavin, but not tryptophan or tyrosine, to CWF light also produces toxic medium. Tryptophan enhances riboflavin-induced phototoxicity, whereas tyrosine diminishes this effect. As photosusceptibility of this system is very high, Fischer’s medium must be fully protected from all sources of light absorbable by riboflavin.

INTRODUCTION

Under controlled conditions, the growth of established mammalian cell cultures is fairly reproducible. In our laboratory, murine leukemia L5178Y has a doubling time of 11.6 ± 0.2 hr and a cloning efficiency in soft agar in excess of 60%. Random disturbances in the growth and cloning efficiency of this cell line cultured in Fischer’s medium prompted an investigation into their cause with the intent of establishing preventive measures. The Fischer’s medium used in our laboratory was prepared from powder by a core tissue culture facility. In the course of its preparation, the medium was not protected from unlimited ambient laboratory light and was then stored in a lighted cold room. It was surmised that the random disturbances in growth and cloning efficiency of L5178Y leukemic cells were due to formation of toxic photoproducts in Fischer’s medium.

Development of phototoxicity in riboflavin-containing medium was originally observed by Warburg et al. (35), who noted that medium containing riboflavin became toxic to mammalian cells when exposed to light and oxygen. Other researchers (17) noted that the combined presence of vitamin and amino acid mixtures was required to produce a phototoxic effect in Eagle's minimal essential medium irradiated with visible light. Development of toxicity in MEM also occurred when riboflavin and tryptophan or riboflavin and tyrosine were present simultaneously during irradiation with near-UV or visible light (29, 32).

MATERIALS AND METHODS

Cell Culture. L5178Y cells in the exponential phase of growth were suspended in F510 (Grand Island Biological Co., Grand Island, N. Y.). The culture was incubated at 37° in the presence of 5% CO2. For growth studies, subcultures were seeded at 10^4 cells/ml in F5 supplemented with 10% horse serum (F5S10). Static subcultures were maintained in 25-sq cm Corning 25100 flasks in a volume of 45 ml/flask. The cells were kept in the exponential phase of growth with dilution of cells to 10^5/ml as required, and growth was monitored for 3 days. Viability was assessed daily by trypsin blue dye exclusion (21) and on Day 1 by cloning in soft agar (3) containing fresh, light-protected F510. Alternatively, exponentially growing cells were cloned directly in F5S10 without prior exposure to F5S10. Laboratory manipulations of cells were performed in ambient CWF light or in very dim indirect incandescent light. All cell counts were done with a hemocytometer.

Medium Preparation. Fischer’s medium was prepared from commercially available powder in core laboratories illuminated with CWF light and stored at 4° in the absence of light. C-medium was prepared under conditions of very low illumination. C-medium, deficient in riboflavin, tryptophan, and tyrosine (FR-Trp-Tyr), was obtained from Grand Island Biological Co. Nutrient-deficient, irradiated medium was supplemented with missing components and serum prior to inoculation with cells. The tyrosine content of powder-derived medium is 60 mg/liter (4); it is 20 mg/liter for C-medium (9). Completely resupplemented, irradiated C-medium was prepared by adding appropriate quantities of the following nutrients: L-arginine-HCl, L-phenylalanine, and L-valine (Sigma Chemical Co., St. Louis, Mo.); L-asparaginase, L-glutamine, L-histidine, L-methionine, L-serine, nicotinamide-HCl, d-pantothenic acid, and thiamine-HCl (General Biochemicals, Chagrin Falls, Ohio); L-cystine-2HCl, L-isoleucine, L-leucine, L-lysine-HCl, L-threonine, and biotin (Nutritional Biochemicals Corp., Cleveland, Ohio); L-tryptophan, L-tyrosine, and riboflavin (P-L Biochemicals, Inc., Milwaukee, Wis.); choline chloride (Schwarz/Mann, Orangeburg, N. Y.); folic acid (Matheson, Coleman, and Bell, Norwood,

1 Supported in part by American Cancer Society Grant CH-35B, the Howard Hughes Medical Institute, and the R. J. Reynolds Medical Oncology Trust Fund.
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Received December 26, 1979; accepted February 19, 1981.
Ohio; \(\textit{i}\)-inositol (Difco Laboratories, Detroit, Mich.); and pyridoxal-HCl (K & K Laboratories, Inc., Plainview, N. Y.). The normal concentration of each component of Fischer’s medium is listed in the “Appendix.” Final concentration of each resupplemented amino acid and vitamin was twice the amount normally present.

Irradiation of Medium. Approximately 300 ml of medium were placed in 500-ml glass bottles. The pH was adjusted to 6.8 and usually rose to a maximum of 7.2 during irradiation. Ambient temperature during irradiation was 25 \(\pm\) 1\(^\circ\). Fischer’s medium was irradiated with a 40-watt CWF lamp (Sylvania F40T12-CW) or with two 15-watt CWF lamps (Sylvania F15T8-CW). The surface of the medium was 13 cm below the lamp. Duration of irradiation time was adjusted to provide an incident near-UV dose at the surface of the medium of 5.2 \(\times\) \(10^4\) J/sq m. Unirradiated control medium samples were wrapped in aluminum foil and placed beside the irradiated aliquots. In experiments comparing relative toxicity induced by several types of light sources, medium was irradiated with two 15-watt CWF lamps, two 15-watt WWXF lamps (Sylvania F15T8-WWX), or two 15-watt GOF lamps (Sylvania F15T8-GO), or it was exposed to very dim indirect, incandescent light. To compare toxicity produced by these alternative light sources, medium was irradiated with WWXF or GOF lamps under identical conditions as were required to provide a dose of 5.2 \(\times\) \(10^4\) J/sq m near-UV light using two 15-watt CWF lamps. The only modification was to place the medium irradiated with dim indirect incandescent light on the laboratory bench top rather than directly below the lamp. The spectral emission of GOF lamps is principally above 500 nm and would not be expected to produce light absorbable by riboflavin. The spectral emissions of CWF and WWXF lamps, however, have a significant portion of the light absorbable by riboflavin. The spectral emissions of CWF and WWXF lamps, however, have a significant portion of the light absorbable by riboflavin.

Near-UV irradiation intensity was measured with a Blak-Ray Model J-221 UV meter (Ultraviolet Products, San Gabriel, Calif.).

RESULTS

Exposure of Fischer’s medium to CWF light corresponding to a dose of 5.2 \(\times\) \(10^4\) J near-UV light per sq m is highly toxic to L5178Y murine leukemia cells (Chart 1). \(F_{nv}\) was supplemented with 10% horse serum and inoculated with \(10^6\) cells/ml. Within 4 hr, cell numbers declined 15%. Cell loss reached 50% after 24 hr, 90% after 48 hr, and 98% after 72 hr. Significant quantities of trypan blue-stained cellular debris were seen as cells continued to undergo lysis throughout the period of observation. The kinetics of development of phototoxicity in Fischer’s medium was determined by measurement of cell viability after inoculation of cells in medium preirradiated with increasing doses of CWF light corresponding to 0.22 to \(5.2 \times 10^4\) J near-UV light per sq m (Chart 2). Doses of 0.87 \(\times\) \(10^4\) J/sq m or less were cytostatic for the first 24 to 48 hr of incubation (Chart 2A) with cells resuming nearly normal growth rate after 48 hr.

Doses greater than 0.87 \(\times\) \(10^4\) J/sq m were cytotoxic. Cell losses were extensive, ranging from 20 to 40% during the first 24 hr of incubation, and were progressive throughout the 78 hr period of observation.

Chart 2B shows a dose-related cytotoxic effect after incubation of cells for 49 or 78 hr in medium which has been preirradiated with CWF light (providing a dose of 0.87 \(\times\) \(10^4\) and 1.73 \(\times\) \(10^4\) J/sq m or less). The chart shows that the maximal degree of toxicity was obtained after approximately 30 hr of irradiation or a dose of 2.2 \(\times\) \(10^4\) J/sq m. Chart 2B also shows that even small doses of CWF light inhibit growth; the deleterious effect of 0.22 \(\times\) \(10^4\) J/sq m is evidenced by growth inhibition observed after 4 hr of subculture in \(F_{mS_{10}}\).

Examination of the 24.5-hr graph (Chart 2B) shows that, at some point between 0.44 and 0.87 \(\times\) \(10^4\) J/sq m, there is a transition from a cytostatic to a cytotoxic effect, which is estimated to be at a dose of 0.66 \(\times\) \(10^4\) J/sq m or 9 hr of irradiation time. A similar examination of the 78-hr graph (Chart 2B) shows that this transition occurs at approximately 1.3 \(\times\) \(10^4\) J/sq m (or 17.5 hr of irradiation time). The difference is attributable to a 48-hr recovery period required for cells incubated in medium irradiated with CWF light, corresponding to a near-UV dose of 0.87 \(\times\) \(10^4\) J/sq m (Chart 2).

In a parallel kinetic experiment, the cloning efficiency of cells incubated in \(F_{mS_{10}}\) for 24 hr was determined (Chart 3). Aliquots of cells were incubated in \(F_{mS_{10}}\) prior to cloning in fresh unirradiated \(F_{S_{0}}\). A dose-related cytotoxic effect was observed.

The simultaneous presence of riboflavin and tryptophan or riboflavin and tyrosine was reported to be necessary for the photochemical formation of toxic products in MEM (29). To
Phototoxicity in Fischer’s Medium

Chart 2: Growth of L5178Y cells in FinS0 as a function of duration of incubation time (A) and dose of CWF light (B). C-medium was irradiated with a CWF lamp. Near-UV doses are related to the hr of irradiation. The unirradiated control was wrapped in aluminum foil and placed beside irradiated samples for 72 hr. Cultures were initiated with 10^5 cells/ml; cell number at 0 incubation time and 0 irradiation is not shown in B; except for this point, data in A are replotted in B.

Chart 3: Surviving fraction and cloning efficiency of L5178Y cells after a 24-hr incubation in FinS0 irradiated with CWF light corresponding to near-UV doses of 0 to 5.2 x 10^4 J/sq m. After a 24-hr incubation in FinS0 (Chart 2A), cells were resuspended in fresh, unirradiated FinS0 and cloned in soft agar. Points, means of 2 experiments, each carried out in quadruplicate. An average of 467 colonies was counted for each dose of CWF light.

As riboflavin sensitizes the photooxidation of the amino acids (histidine, methionine, tryptophan, and tyrosine) and possibly some vitamins (17, 30), it is necessary to establish whether the simultaneous presence of riboflavin and tryptophan or riboflavin and tyrosine also provides determinants for development of phototoxicity to L5178Y cells in Fischer’s medium, medium lacking these 3 components (FR-Trp-Tyr”) was irradiated with CWF light. Aliquots of this medium deficient in riboflavin, tryptophan, and tyrosine were also supplemented with each missing component, singly or in various combinations at the concentration prescribed for Fischer’s medium and irradiated (Chart 4; Appendix). After irradiation, components omitted during irradiation with CWF light were added; the final concentration of each component was within 2% of that normally present. Each medium was supplemented with 10% horse serum, and growth was observed after inoculation with 10^5 cells/ml. Viable cell counts, taken after 4, 24, 54, and 74 hr of incubation, show that cessation of growth and cytotoxicity occur when riboflavin alone is present during irradiation (FR-Trp-Tyr”). Irradiation of medium lacking riboflavin (FR-Trp-Tyr or FR-Trp-Tyr”) produced neither cytostasis nor cytotoxicity for the L5178Y cells. A rapid cell loss occurring within 4 hr after inoculation was observed only when riboflavin and tryptophan were present during irradiation.

The possible coparticipation of riboflavin, tryptophan, and tyrosine in the development of phototoxicity was further assessed by determining the clonogenic fraction of cells incubated in each of the irradiated media described in Chart 4 (Chart 5). In all conditions in which riboflavin is present during irradiation with CWF light, a toxic photoproduc was produced which resulted in a substantial reduction in the cloning efficiency of L5178Y cells; an incubation time of 4 hr was sufficient to reduce cloning efficiency by 15 to 30%. The coparticipation of tryptophan and tyrosine in the development of riboflavin-mediated phototoxicity is shown by the difference in cloning efficiencies after a 54-hr incubation in irradiated medium. The cloning efficiency of cells incubated in medium which contained riboflavin (FR-Trp-Tyr”) during irradiation declined 2 orders of magnitude. While the presence of tyrosine during irradiation of medium containing riboflavin (FR-Trp-Tyr”) provided protection from phototoxicity, the presence of tryptophan and riboflavin (FR-Trp-Tyr”) during irradiation resulted in a 10-fold increase in phototoxicity which was not overcome by the presence of tyrosine (FR-Trp-Tyr”). Irradiation of medium deficient in riboflavin but containing either tryptophan (FR-Trp-Tyr”) or tyrosine (FR-Trp-Tyr”) has no apparent phototoxic consequences.

As riboflavin sensitizes the photooxidation of the amino acids (histidine, methionine, tryptophan, and tyrosine) and possibly some vitamins (17, 30), it is necessary to establish whether
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Chart 4. Growth of L5178Y cells in FhVs0: effect of presence of riboflavin and tryptophan (A) or riboflavin and tyrosine (B) during irradiation with CWF light on the extent of phototoxicity. C-medium lacking riboflavin, tryptophan, and tyrosine was supplemented with appropriate quantities (see "Appendix") of riboflavin, tryptophan, and tyrosine, singly or in combination, and irradiated 72 hr with CWF light to give a dose of $5.2 \times 10^5$ J per sq m. Fh, was then supplemented with the missing nutrients and 10% horse serum. FhvS,0 was inoculated with $10^5$ cells/ml, and growth was observed. The data are the average from 2 experiments. O, unirradiated control; O, Fh, R*Trp*Tyr*; △, Fh, R*Trp*Tyr*; ○, Fh, R*Trp*Tyr*; □, Fh, R*Trp*Tyr* (A) or Fh, R*Trp*Tyr* (B); ■, Fh, R*Trp*Tyr* (A) or Fh, R*Trp*Tyr* (B).

Chart 5. Cloning efficiency of L5178Y cells following incubation in FhVs0 containing riboflavin, tryptophan, and tyrosine, singly or in combination, during irradiation. Aliquots of cells from each condition for the time points 4, 24, and 54 hr of incubation time (Chart 4) were resuspended in fully supplemented unirradiated medium and cloned in soft agar. All cloning efficiencies are normalized to that of the unirradiated control. Numbers in parentheses, number of colonies observed for each point. Where no colonies were found, a dashed line is drawn to the point corresponding to a single colony. O, R*Trp*Tyr*; △, R*Trp*Tyr*; ○, R*Trp*Tyr*; □, R*Trp*Tyr*; ▲, R*Trp*Tyr*; ■, R*Trp*Tyr*.

nutritional deprivation accounts for the observed cytotoxicity of Fh, After irradiation, aliquots of Fh, medium were supplemented with a complete amino acid mixture, a complete vitamin mixture, or a complete vitamin plus amino acid mixture. The final concentration was twice that normally present in Fischer's medium (see "Appendix"). After further supplementation with 15% horse serum, cells were cloned directly in irradiated, resupplemented medium. All manipulations other than irradiation of the medium were performed in dim light. The data in Table 1 show that cells cloned in Fh, fail to survive irrespective of the postirradiation supplementation, the cloning efficiency in each Fh, being less than 0.001% at a cell density of $10^5$ cells/15 ml of cloning medium.

Ambient laboratory light is frequently supplied by CWF lamps. These lamps emit near-UV and visible light at the 373- and 445-nm absorption bands for riboflavin (39) and are thus able to produce riboflavin-sensitized photooxidation reactions. Laboratory areas used for cell culture, preparation, and storage of Fischer's medium were surveyed for ambient near-UV light exposure (Table 2). Near-UV radiation was found at all locations. The relative intensity of light emitted at 373 and 445 nm was estimated from the ratios of the spectral output at 365, 373, and 445 nm of the light sources (39). Examination of the spectral output of other fluorescent lamps (39) showed that WWXF lamps emit less light absorbable by riboflavin than do CWF lamps. GOF lamps show no emission of light below 500 nm, except a weak mercury line at 365 nm.

To compare the relative degree of phototoxicity produced in Fischer's medium by different lamps, C-medium was prepared in dim incandescent light, and aliquots were irradiated with two 15-watt lamps of the following types: CWF; WWXF; GOF; or very dim incandescent. The dose of near-UV light from the CWF lamps was $5.2 \times 10^5$ J/sq m. After irradiation, Fischer's medium was supplemented with 10% horse serum and inoculated with $10^5$ cells/ml for growth studies (Chart 6) or with 15% horse serum for cloning studies (data not shown). All manipulations were performed in very dim, indirect incandescent light. The medium irradiated with CWF light is highly toxic, the cell numbers declining throughout the 2-day period of observation. Medium irradiated with WWXF light was nearly, but not completely, cytostatic during the first day. The doubling times for cells subcultured in medium irradiated with WWXF light show partial recovery during the second day, the doubling time

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declining from 52 hr the first day to 19.9 hr on Day 2. Irradiation of Fischer's medium with GOF light resulted in neither cytostasis nor cytotoxicity for cells cultured in this medium.

Comparison of viability after irradiation with CWF, WWXF, GOF, or very dim, indirect incandescent light shows a similar relationship of phototoxicity to spectral output as is observed in growth studies (data not shown). Medium irradiated with CWF was the most toxic, giving a cloning efficiency of less than 0.001% at a cell density of $10^5$ cells/15 ml cloning medium. The cloning efficiency of cells in Fh«Si5 prepared from medium exposed to CWF light is shown to be highly toxic to murine leukemia L5178Y. This result is consistent with earlier observations (17, 29, 35) of the toxic effects of light-exposed medium on other mammalian cell lines. Doses of CWF light corresponding to bench-top exposure to ambient CWF light for as little as 3 hr produce measurable amounts of phototoxicity in Fischer's medium. Other significant sources of light absorbable by riboflavin include incandescent lamps and sunlight (39).

### DISCUSSION

Fischer's medium exposed to CWF light is shown to be highly toxic to murine leukemia L5178Y. This result is consistent with earlier observations (17, 29, 35) of the toxic effects of light-exposed medium on other mammalian cell lines. Doses of CWF light corresponding to bench-top exposure to ambient CWF light for as little as 3 hr produce measurable amounts of phototoxicity in Fischer's medium. Other significant sources of light absorbable by riboflavin include incandescent lamps and sunlight (39).

#### Phototoxicity in Fischer's Medium

The survival curve of cells subcultured in $F_{h1}S_{10}$ is triphasic, with an initial shoulder indicating that damage is incurred by cells in lightly irradiated medium. The sharp change in slope which occurs at $0.87 \times 10^4$ J/sq m marks the transition from cytostasis to cytotoxicity in growth experiments (Chart 2B). This is also the threshold dose for the lytic effect of phototoxic medium. It is suggested that lysis, as an additional form of cumulative damage, selectively removes a subpopulation of heavily damaged cells. Selective depletion of the more highly damaged cells by lysis means that the data for the first portion of the survival curve relate to the whole population of cells, whereas the second phase assesses the remaining unlysed subset. Thus, estimation of the clonogenic fraction in this phase of the curve is an underestimate of medium-induced damage. The effect of Fischer's medium irradiated with CWF light on L5178Y cells differs in several aspects from the effect of MEM irradiated with "daylight" fluorescent light (32) on D98/AH, 3T6-DF8, and V79 cells. Wang (32) found that MEM irradiated for 24 hr was not toxic to these cell lines. In contrast, L5178Y cells, incubated in Fischer's medium irradiated with CWF light for much shorter periods, showed dose-dependent cytostatic and cytotoxic effects (Charts 2 and 3). Stoien and Wang (29) also report little or no toxicity when riboflavin is present, and tryptophan and tyrosine are deleted during irradiation of MEM with near-UV light. We find a substantial degree of phototoxicity in Fischer's medium when riboflavin is present and tryptophan and tyrosine are deleted (Charts 4 and 5). A further difference is that tyrosine does not contribute to the phototoxicity of irradiated Fischer's medium as was reported for MEM (29). Instead, the presence of tyrosine in Fischer's medium is photoprotective (Chart 5).

These differences may be attributed to differences in one or more of the following factors: varied sensitivity of the cell lines to toxic photoproducts; concentration of photoactive medium components; oxygen concentration during irradiation; possible presence of pyruvic acid in MEM during irradiation; and possible differences in pH during irradiation, a higher pH favoring photodegradation of tyrosine (28).

As DNA is a cellular target for medium-mediated toxic photoproducts (6, 10) which produce both single-strand breaks (1, 10, 33) and DNA-protein cross-links (6), L5178Y cells may have a diminished repair capacity relative to the mouse cell line 3T6-DF8 studied by Wang (32) and Wang and Nixon (34). The crucial role of proficient DNA repair mechanisms in the determination of sensitivity by mammalian cells to the toxic trypto-

### Table 1

Cloning efficiency of L5178Y cells in irradiated complete Fischer's medium, effect of postirradiation supplementation with vitamins and amino acids

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Postirradiation supplementation</th>
<th>Cell inoculum/flask</th>
<th>Colonies/flask</th>
<th>% of cloning efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>None</td>
<td>100</td>
<td>65.8</td>
<td>65.8</td>
</tr>
<tr>
<td>None</td>
<td>Complete vitamins</td>
<td>100</td>
<td>67.9</td>
<td>67.9</td>
</tr>
<tr>
<td>None</td>
<td>Complete amino acids</td>
<td>100</td>
<td>58.6</td>
<td>58.6</td>
</tr>
<tr>
<td>None</td>
<td>Complete vitamins and amino acids</td>
<td>100</td>
<td>67.4</td>
<td>67.4</td>
</tr>
<tr>
<td>Irradiated</td>
<td>None</td>
<td>$10^3$</td>
<td>None</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Irradiated</td>
<td>Complete vitamins</td>
<td>$10^3$</td>
<td>None</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Irradiated</td>
<td>Complete amino acids</td>
<td>$10^3$</td>
<td>None</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Irradiated</td>
<td>Complete vitamins and amino acids</td>
<td>$10^3$</td>
<td>None</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*See "Appendix" for concentrations of components of Fischer's medium.

### Table 2

Ambient near-UV radiation in areas used for preparation and storage of Fischer's medium and for cell culture

<table>
<thead>
<tr>
<th>Room function</th>
<th>Type of light</th>
<th>Survey site</th>
<th>Near-UV intensity (microwatt/sq cm)</th>
<th>Near-UV dose/hr ($10^{-2} \times J$/sq m)</th>
<th>Estimated hourly dose of light absorbable by riboflavin ($10^{-2} \times J$/sq m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium preparation</td>
<td>CWF</td>
<td>Bench top</td>
<td>20</td>
<td>10.8</td>
<td>10.8</td>
</tr>
<tr>
<td>Medium storage 1</td>
<td>Incandescent</td>
<td>Shelf 1</td>
<td>30</td>
<td>10.8</td>
<td>10.8</td>
</tr>
<tr>
<td>Medium storage 2</td>
<td>CWF</td>
<td>Bench top</td>
<td>30</td>
<td>10.8</td>
<td>10.8</td>
</tr>
<tr>
<td>Warm room</td>
<td>CWF</td>
<td>Bench top</td>
<td>20</td>
<td>10.8</td>
<td>10.8</td>
</tr>
<tr>
<td>Medium storage 1</td>
<td>CWF</td>
<td>Shelf 2</td>
<td>50</td>
<td>18.0</td>
<td>18.0</td>
</tr>
<tr>
<td>Medium storage 2</td>
<td>CWF</td>
<td>Shelf 2</td>
<td>20</td>
<td>10.8</td>
<td>10.8</td>
</tr>
</tbody>
</table>
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**Chart 6. Growth of L5178Y cells in Fm* Sio. Comparison of phototoxicity produced by irradiation with fluorescent lamps of differing spectral output.** C-medium was prepared in dim incandescent light. Aliquots were irradiated 16 hr with two 15-watt CWF lamps, two 15-watt WWXF lamps, or two 15-watt GOF lamps. The incident near-UV dose at the surface of the medium exposed to the CWF lamps was 5.2 X 10^4 J/sq m. Medium was supplemented with 10% horse serum and inoculated with 10^5 cells/ml. Cells grown in unirradiated medium or medium irradiated with GOF light were diluted to 10^5 cells/ml after 1 day of incubation.

O, unirradiated control; □. CWF; □, WWXF; GOF.

The formation of photoproducts toxic for L5178Y leukemic cells, manuscript in preparation.

Interaction (type 1 process) (5, 14), singlet O2 pathway (type 2 (15, 16, 31) yields the highly oxidizing species, singlet oxygen. Singlet oxygen readily oxidizes histidine, methionine, and tryptophan (18, 25). Photooxidation of tryptophan and methionine would be expected to yield toxic photoproducts (10, 34, 38). It is suggested that, in the case of MEM, tryptophan is functioning as its own photoprotective agent by competing with O2 in quenching the phosphorescence of the riboflavin triplet, similar to a reaction observed by Nilsson et al. (25). As Fischer’s medium contains more riboflavin and less tryptophan than does MEM, we suggest that Fischer’s medium is more prone to generation of toxic photoproducts than is MEM.

The lack of toxicity due to riboflavin-sensitized photooxidation of tyrosine is possibly due to the differences in pH during irradiation. The pH of Fischer’s medium in the experiments reported in this paper was 6.8 at the start of irradiation and rose to a maximum of 7.2 during the course of irradiation. As only the phenolate form of tyrosine is readily photooxidizable, the rate increasing as pH increases from 7 to 9.5 (28, 37), irradiation at pH 7.0 ± 0.2 would not be expected to yield a significant quantity of tyrosine photoproducts. Rather than augment phototoxicity of irradiated Fischer’s medium, tyrosine at neutral pH is partially photoprotective (Charts 4 and 5). The basis for the photoprotection is postulated to be complex formation between riboflavin and tyrosine observed in buffered solutions at pH 7 (19, 27). The expected photochemical consequence of complex formation is diminished riboflavin-sensitized photooxidation of tryptophan (19).

Another possible variable is that the MEM used by Stoin and Wang (29) and Wang (32) contained sodium pyruvate, an effective trapping agent for peroxides (38), which would detoxify a portion of the H2O2 produced by photooxidation of tryptophan (10, 20, 34, 38).

Preliminary experiments6 indicate that lysis, occurring shortly after cells were suspended in Fm* Sio, is prevented by postirradiation treatment with catalase. Viability studies of these cells indicate that H2O2 participates in lysis of cells in concert with another lethal tryptophan photoprotuct.

Photooxidation could conceivably deplete Fischer’s medium of essential nutrients (28). The data in Table 1 show that depletion of essential nutrients during irradiation is not the cause of growth failure and support the hypothesis that the cells die as a result of exposure to toxic photoproducts.

Because the test system, L5178Y-Fischer’s medium, is highly sensitive to the presence of toxic photoproducts, the ability of several light sources to produce toxic effects was compared (Chart 6). Cells suspended in Fischer’s medium irradiated with CWF or WWXF light showed growth failure and low cloning efficiencies, indicative of phototoxicity. The lesser effectiveness of WWXF light in producing phototoxity is consistent with its lower output of light absorbable by riboflavin. As expected, GOF light did not produce photooxidation as an insignificant amount of light is emitted below 500 nm.

As leakage of light may occur at the lamp ends or at defects in the lamp phosphor, we recommend the use of yellow sleeve filters with end caps on conventional laboratory fluorescent lamps. Light below 500 nm is excluded by these filters (13). The lack of phototoxicity in medium irradiated with very dim, indirect incandescent light is attributable to the low dose of light produced.

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