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Letter to the Editor

Phototoxicity of *N*-2-Hydroxyethylpiperazine-*N'*-2-ethanesulfonic Acid-buffered Culture Media for Human Leukemic Cell Lines[1]

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Several investigators have shown previously that, due to exposure of certain culture media to near-UV or visible light, products are formed that are toxic for cells in culture (1, 3, 4). Compounds involved in light-induced toxicity include riboflavin, riboflavin-tryptophan, and riboflavin-tyrosine (1, 2, 4). Together with O_2, these components, either alone or in complexes, form, amongst others, toxic peroxides (4).

Here, we report experiments with the RPMI-1788 (normal B-cell) and Molt-4 (leukemic T-cell) lines. Both cell lines were cultured in RPMI 1640 DM[2] medium, supplemented with 10% fetal calf serum (v/v), penicillin (100,000 units/liter), and streptomycin (100,000 μg/liter) in 25- or 75-sq cm plastic culture flasks or in 2-ml cluster dishes. RPMI 1640 DM medium (Flow Laboratories, Irvine, Ayrshire, Scotland) contains HEPES (4,770 mg/liter), NaCl (6,400 mg/liter), and $NaHCO_3$ (1,000 mg/liter), whereas RPMI Medium 1640 is a medium without HEPES containing NaCl (6,000 mg/liter) and $NaHCO_3$ (2,000 mg/liter). Both media contain other constituents in identical concentrations. The culture medium was exposed to light in closed 25-sq cm polystyrene culture flasks (Catalogue No. 25100; Corning Glass Works, New York, NY) placed horizontally on dull black paper at 25° immediately before use. The fluorescent tubes (Philips TL34) in use in our laboratory at that time are comparable to the "cool white" fluorescent tubes regularly used (1).

In experiments in which the dose effect of HEPES was studied, RPMI 1640 DM was also used, however, with different concentrations of HEPES (H-3375; Sigma Chemical Co.) added. The cells were grown at 37° in a water-saturated atmosphere containing 2.5% CO_2. Cultures were initiated at 3×10^5 cells/ml. After 40 hr of culture, the number of viable (trypan blue exclusion) cells was counted (Burker-Türk chamber). Under the conditions used, the pH of the cell cultures was stable at pH 7.3 ± 0.1 (S.D.). When no HEPES was used, the $NaHCO_3$ in the medium and the 2.5% CO_2 atmosphere had sufficient buffering capacity.

Our first observations were that, when cells were cultured in HEPES-buffered RPMI 1640 DM medium, exposed to daylight or "Philips TL34 light" for relatively short periods of time prior to use, cell death occurred. In Molt-4 cultures grown in medium that had been exposed to daylight for less than 1 hr, total cell death was observed within 24 hr of culturing.

In order to quantify the phototoxic effects of daylight, we used a "Philips-TL57 daylight lamp." Light doses were measured with a Parvux 2 luxmeter (1 lux·hr = 529 J/sq m). Molt-4 cultures (T-cells) turned out to be more sensitive to light-exposed HEPES-buffered medium than RPMI-1788 cultures (B-cells); 1000 lux·hr prevented the Molt-4 cells in culture from growing (*i.e.*, the number of cells did not increase), whereas, for RPMI-1788 cells, 6000 lux·hr were needed to achieve the same effect. Higher exposure doses resulted in cell death (*i.e.*, the number of cells in culture decreased). A dose of 2000 lux·hr on the medium killed all Molt-4 cells within 40 hr. This dose of 2000 lux·hr is only about twice the dose of light which is reached under normal laboratory conditions. In the absence of 20 mM HEPES, 2000 lux·hr caused no marked inhibition of growth in Molt-4 cell cultures. Therefore, HEPES seemed to be involved in light-induced toxicity of the RPMI 1640 DM medium.

In order to further characterize the role of HEPES in this phenomenon, we initiated Molt-4 cell cultures in RPMI 1640 DM containing different concentrations of HEPES. The culture medium was exposed to the artificial daylight (2750 lux·hr) prior to use. In Chart 1, it is shown that Molt-4 cell growth is affected even at relatively low HEPES concentrations. At HEPES concentrations of 1 and 3 mM, cell growth was clearly inhibited. At higher concentrations, cell death occurred.

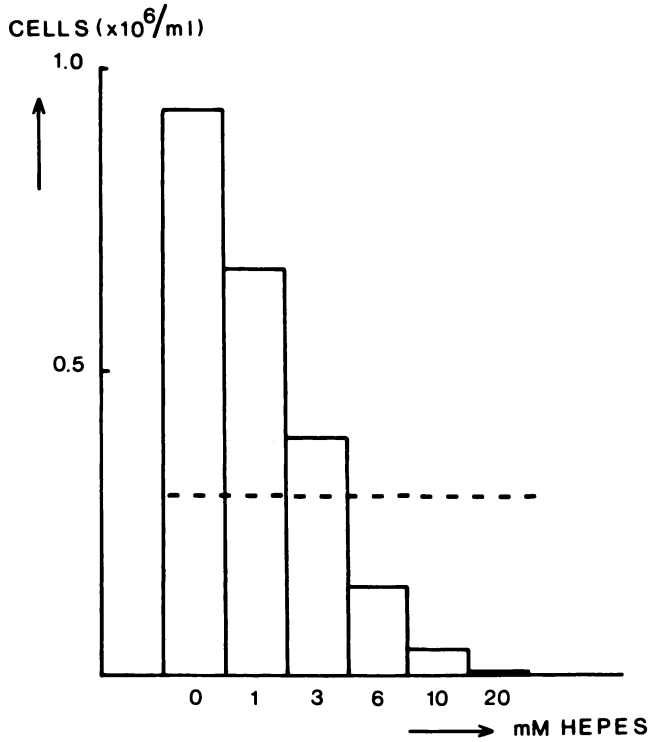

Chart 1. Number of viable cells in culture after 40 hr of culturing in RPMI 1640 DM medium containing different concentrations of HEPES. Data represent a typical experiment. The medium was exposed to 2750 lux·hr prior to use. - - - -, cell concentration at which the cultures were initiated (0.3×10^6 cells/ml).

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[2] The abbreviations used are: DM, Dutch modification; HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.

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In an experiment in which the dose effect of light was studied, it was shown that, at 10 mM HEPES, 500 lux·hr had no marked effect on Molt-4 cell growth. Light doses from 1000 to 3000 lux·hr caused a gradual decrease of viable cells in culture after 40 hr. When the medium had been exposed to light doses higher than 3000 lux·hr, complete cell death was observed.

From the experiments described above, we believe that HEPES plays a role in phototoxicity of the RPMI 1640 DM medium. The mechanism of the phototoxicity of HEPES has not been the subject of this study and has still to be elucidated. Whether the effect of HEPES is direct or indirect remains to be seen. HEPES may act as a catalyst in the formation of peroxides from riboflavin or other compounds in the medium.

The toxic effect of light on RPMI 1640 DM medium can be eliminated completely if 2 mM sodium pyruvate are added to the culture. Sodium pyruvate has been used previously as a trapping agent for peroxides (5).

The HEPES-buffered RPMI 1640 DM medium is widely used for *in vitro* studies on normal and leukemic cells. Therefore, when performing culture experiments, the phototoxic effects mentioned above should be taken into consideration.

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