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Letter to the editors

## Toxicity of light-exposed Hepes media

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Dear Editors,

In view of the extensive use of Hepes-buffered media in studies involving cell cultures, we would like to call the attention of the readership of the journal to the information we (Zigler et al., 1985) and others (Spierenburg et al., 1984) have collected concerning the adverse effects of Hepes-containing media following exposure to light.

RPMI 1640 medium supplemented with Hepes at 25 mM became toxic to thymocyte cultures following exposure to fluorescent light at 2 mW/cm<sup>2</sup> for as little as 30 min; exposure for 3 h reduced thymocyte responses in culture by > 99%. Medium with no Hepes had minimal or no toxic effects following similar exposures. The results of these experiments indicate that exposure of Hepes-buffered media to the bright illumination commonly used in laminar flow hoods could lead to toxic effects on cells subsequently cultured in the medium. While such effects would not generally be comparable to the extreme cytotoxicity observed in our experiments, our observations indicate that unacceptable levels of variability in the responses of cell cultures can result from failure to

protect the medium from light during routine laboratory procedures. Our data have further demonstrated that the toxicity of light-exposed medium is due to formation of hydrogen peroxide in amounts directly related to the light exposure.

The data thus indicate the necessity to protect Hepes-containing media from exposure to light. Wrapping medium bottles with aluminum foil was found a simple and effective measure. It should also be mentioned that hydrogen peroxide levels slowly decrease upon storage in darkness, thus reducing gradually the toxicity of light-exposed media.

### References

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- Zigler, Jr., J.S., Lepe-Zuniga, J.L., Vistica, B. and Gery, I. (1985) Analysis of the cytotoxic effects of light-exposed Hepes-containing culture medium. *In Vitro Cell Dev. Biol.* 21, 282.

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