

Estrogenic Effects of Uranium in Human Breast Cancer Cells

Gillian Generoso¹, Stefanie Raymond-Whish², Karen Chase², and Cheryl A. Dyer²

(¹Dept. of Genetics at Rutgers University, New Brunswick, NJ ²Dept. of Biological Sciences at Northern Arizona University, Flagstaff, AZ)

Abstract

Although it has long been known that uranium's radioactive properties cause numerous health problems, its role as an endocrine-disrupting chemical has yet to be well understood. In this study, we investigated uranium's ability to mimic estrogen by stimulating growth in human breast cancer cells. After exposing cells to estrogen, uranium, or only media, we found that uranium elicited estrogenic responses. However, as the results show, these responses may depend on the duration of exposure and/or uranium concentration.

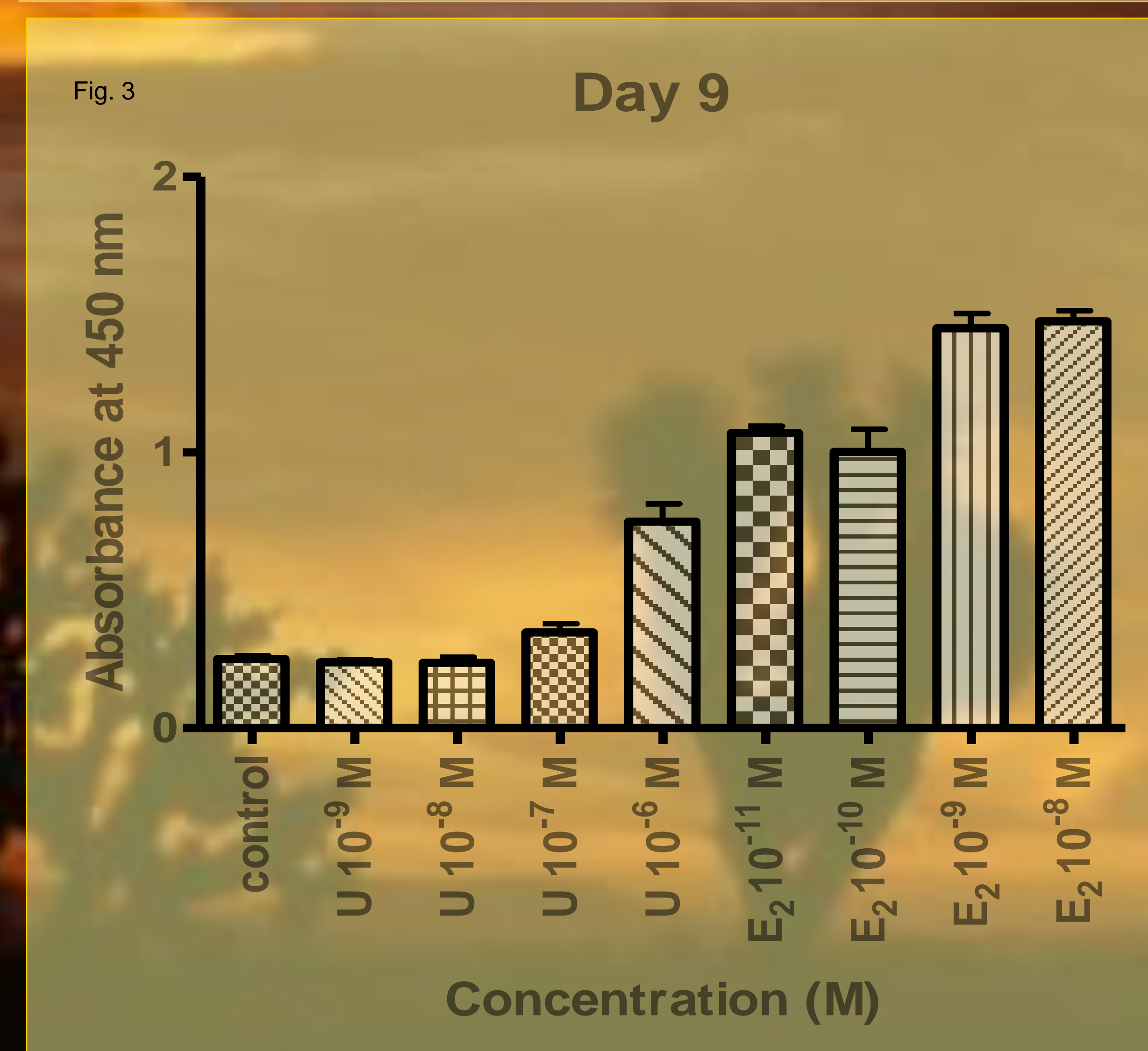
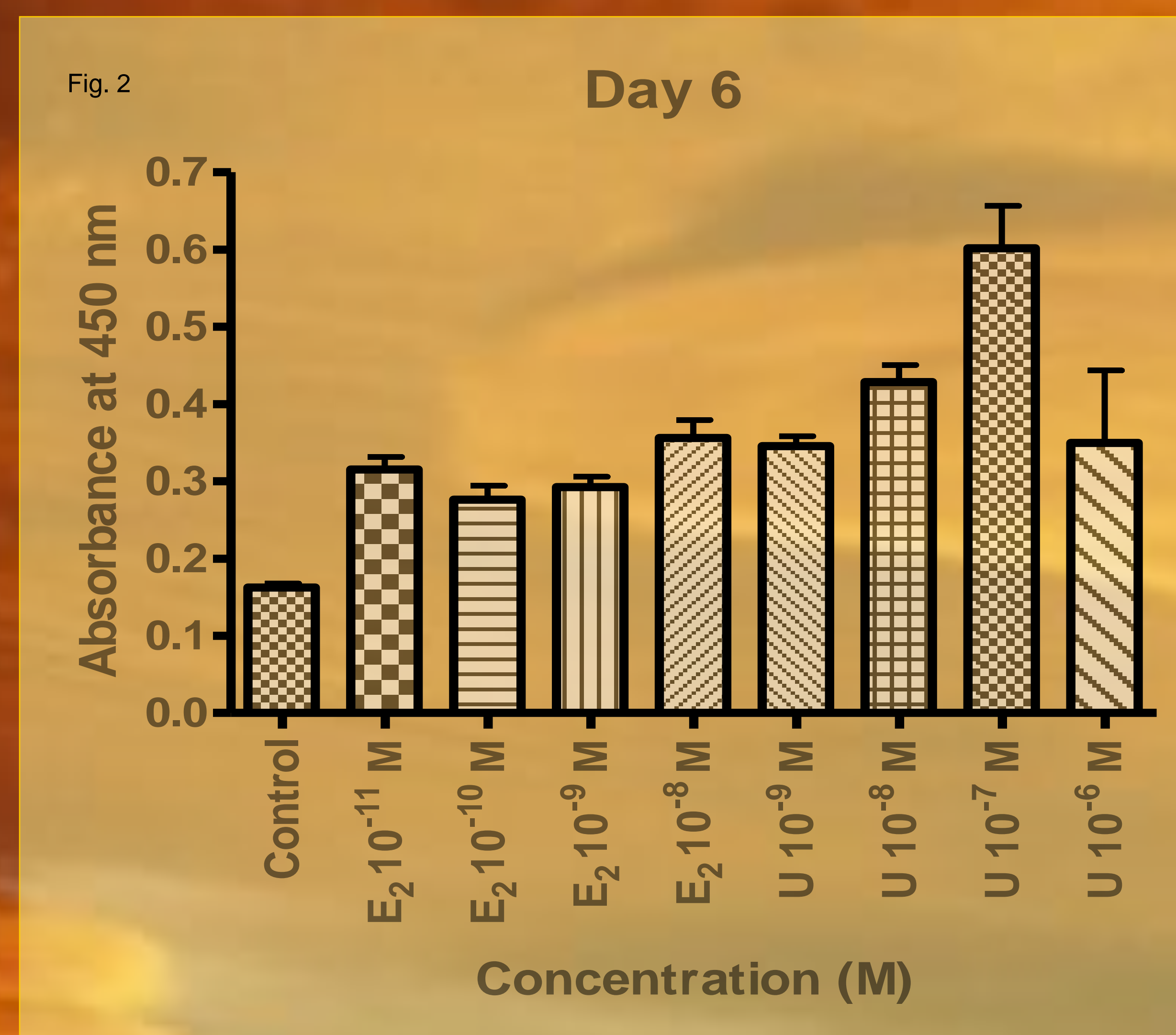
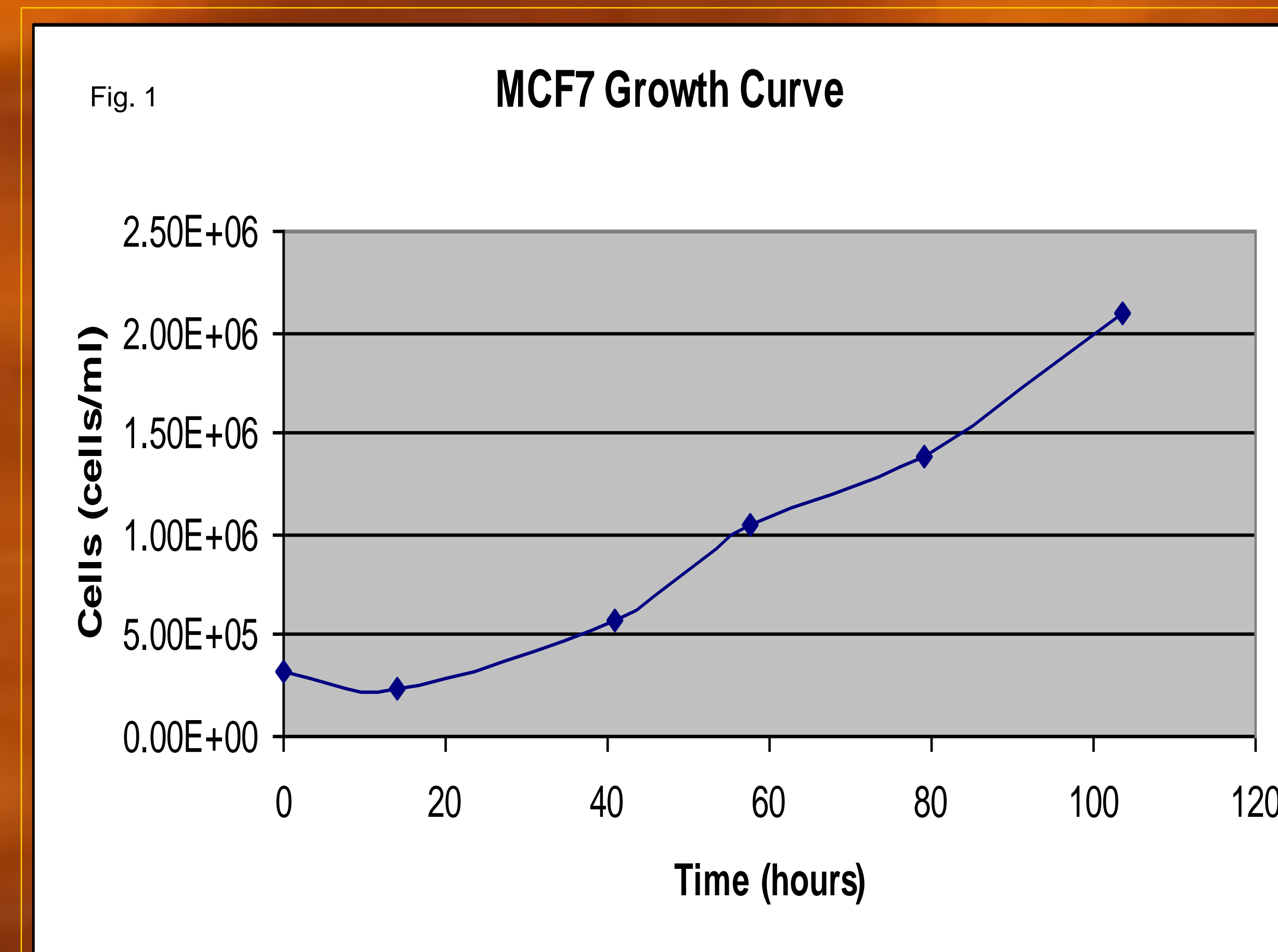
Introduction

The adverse effects of uranium to human health have historically been well-studied, as the boom in uranium mining from the 1940s to the 1980s resulted in respiratory diseases in hundreds of miners (Panikkar and Brugge, 2007). However, much of this research has been devoted to uranium's radioactive properties and not to its properties as an endocrine disruptor. In a pioneer study by Maynard and Hodge in 1949, the permanent impact of uranium as an endocrine-disrupting chemical was established after uranium-consuming female rats showed significant weight loss, reduced litter numbers, and irregular estrous cycles. In this study, we aimed to determine the estrogenic effects of uranium on human breast cancer cells. By growing cells in culture, we sought to determine whether uranium elicits responses similar to those brought about by estrogen.

Materials and Methods

For all experiments, we used the MCF7 human breast cancer cell line. We initially grew three separate cultures over a period of seven days in order to produce an average growth curve and establish a generation time. The cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) with insulin, penicillin-streptomycin solution, and fetal bovine serum. Cell numbers were obtained using Trypan Blue to stain the cells and a hemocytometer. Through this technique, we were able to produce a growth curve and determine the doubling time of the cells.

Once the doubling time was known, we measured cell proliferation as the cells were being exposed to estradiol (E₂) and uranium. For this, we used a WST-1 assay (Berridge et al., 1996). The cells were treated with very low concentrations of E₂ and uranium diluted in Minimum Essential Media (MEM) α . The treatments were done every two days for nine days. Measurements were taken on Day 6 and Day 9 by measuring light absorbance of the assay. The assay involved the use of the WST-1 reagent to dye the mitochondria, and therefore, the proliferation of the cells. As the reagent oxidized the mitochondria, the clear media in which the cells were suspended turned into a yellow color. Absorbance of the yellow color was then measured using the SpectraMax Plus.



Results

Our initial experiment shows exponential cell proliferation without estradiol or uranium treatments (Figure 1). Whereas the seeding density was about 3.0x10⁵ cells, the number grew to over 2 million cells in a matter of 103 hours, with a generation time of approximately 20 hours.

On Day 6 of the subsequent experiment, both E₂ and uranium data show greater cell proliferation than the control (p<0.0001, n=12). Moreover, uranium data shows significantly greater proliferation than E₂ data (p<0.0001, n=12). (Figure 2)

The collection at Day 9 shows significance for cell proliferation due to E₂ (p<0.01, n=12). However, uranium-mediated cell proliferation was only significant for the 10⁻⁶ M concentration (p<0.01, n=12). (Figure 3)

Conclusion

E₂, a naturally occurring chemical within the human body, is a form of estrogen that uses the estrogen receptor to make cells grow (Dahlman-wright et al., 2006). In this study, we attempted to determine if uranium can mimic estrogen's effect on the cells. Results from Day 6 suggest that uranium can, in fact, exhibit and even augment estrogenic effects of cell proliferation. However, data from Day 9 suggests that uranium in concentrations other than 10⁻⁶ M has no such effects. Previous studies have shown, however, that uranium did, in fact, mimic the E₂ stimulated growth of cells (Raymond-Whish and Dyer, 2006).

In the next phase of our project, we will utilize specific estrogen receptor (ER) antagonists to determine whether uranium-mediated responses in the cell depend on alpha, beta, or both types of receptors to increase cell number.

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Contact: gilllian@eden.rutgers.edu

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