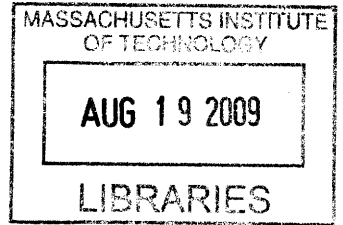


**Effect of Continuous Irradiation on Proliferation of
CHO-K1 and xrs-6 cells under Low and High
Oxygen Conditions**

by

An T. Vu



Submitted to the Department of Nuclear Science and Engineering
in partial fulfillment of the requirements for the degree of

Bachelor of Science in Nuclear Science and Engineering

at the

MASSACHUSETTS INSTITUTE OF TECHNOLOGY

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ARCHIVES

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Abstract

The goals of this research were to determine: 1) the effect of continuous irradiation on Chinese Hamster Ovary cells, CHO-K1 (wild type) and xrs-6 cells (mutant), and 2) the effect of continuous irradiation under low (5%) and high (20%) ambient oxygen conditions. Other authors have found continuous irradiation at low dose rates to have beneficial effects on cells. Oxygen is known to be harmful to DNA as it induces free radicals. The CHO-K1 cells were continuously irradiated for 7 weeks on Am²⁴¹ foils at a photon dose rate of $1.76 \pm 0.02 \frac{\text{cGy}}{\text{hr}}$. The xrs-6 cells were irradiated for one week on the same foils. Using the CyQUANT assay, the CHO-K1 cells showed no statistically significant difference in proliferation between the irradiated and control cells, even for total accumulated doses (> 20 Gy) that are lethal when delivered acutely. The same result was observed with the xrs-6 cells. However, when plated in flasks as opposed to well-plates, the cells showed lower rates of proliferation when irradiated, by a factor of 2 for CHO-K1 and by a factor of 50 to 100 for xrs-6. There was no significant difference in proliferation of both cells lines in the low and high oxygen conditions.

Thesis Supervisor: Jacquelyn C. Yanch
Title: Professor

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I would like to give a particular note to Matthew Davidson and Dwight Chambers to whom I turned to on a weekly basis. I cannot thank both of them enough for their relentless help through this project. Their willingness to help with setting up and maintaining my experiments as well as immediate responses whenever something went amiss were truly appreciated.

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Chapter 1

Introduction

The biological effects of low doses and low dose rates of ionizing radiation have been heatedly debated for over a century. The biological effects of high doses are well documented from follow-up studies of people exposed to high doses such as atomic bomb survivors.[1, 2] High doses of ionizing radiation are harmful to humans as they are known to cause cancer and other deleterious effects. The understanding of the biological effect of low doses of radiation is currently derived from linear extrapolations of data gathered from the high dose radiation epidemiological studies.[1, 3] This model of extrapolation, known as the Linear-No-Threshold (LNT) model, assumes that all levels of radiation are harmful.[1, 2] However, more evidence seems to indicate that low levels of radiation could be helpful, and that the LNT model overestimates the harmful biological effects at low doses.[2, 4] Studies have shown that at low-dose rates, those somewhat higher than background rates, induce beneficial biological effects.[5, 6] A thorough understanding of the effects of low doses and low dose rates of radiation is crucial as an overestimate means that resources are unnecessarily spent on protection, while an underestimate means that human health is unknowingly endangered.

1.1 Background

Beneficial effects of low doses and low dose rates of ionizing radiation have been observed and studied in depth. This section will provide a brief background on the

current findings of a radiation adaptive response and its relation to continuous low dose rates of radiation.

1.1.1 Adaptive Response

The adaptive response is the ability of an organism to better handle stress from an agent by previous exposure to a smaller amount of that agent.[7] In the case of radiation induced stress, the adaptive response has been widely documented. Extensive studies on different cell lines have indicated the evidence of radiation adaptive response. For example, preconditioned cells (cells exposed to a low dose of radiation) incurred less DNA damage, fewer mutations, and were better able to survive under acute radiation exposure than their control counterparts.[8, 5, 9] Studies on mice have shown to reduce occurrence of cancers and increase life expectancy when the mice are exposed to low levels of radiation.[6, 10, 11] The adaptive response is a sign that not all radiation exposure is harmful and that it can be beneficial at low doses. Henceforth, more studies need to be conducted to increase our understanding of the effect of radiation at low doses and low dose-rates. The current low-dose radiation model based on the LNT model will need to be reconsidered based on current and future findings, especially since it is the basis for most current day radiation protection and regulation.[3]

1.1.2 Continuous Low-Dose-Rates

Most adaptive response studies use a single condition dose of a few cGys.[12, 13, 14, 5, 10] However, since humans are mostly exposed to continuous low dose-rates of radiation, it is important to understand how such irradiations affect the radiosensitive behavior of cells and tissues.

Continuous irradiation experiments are often performed using mice. A study by Bhattacharjee indicated that continuous 5-day irradiation on mice significantly reduced the number of thymic lymphomas induced in the preconditioned mice compared to the control mice. The mice were irradiated at a rate of $1 \frac{\text{cGy}}{\text{day}}$ from Co-60 and

challenged 24 hour after precondition at 3 Gy. The number of thymic lymphomas was recorded at 15, 30, 60, 90, and 210 days after challenge exposure. For control mice, thymic lymphomas were found at all time intervals. For preconditioned mice, no thymic lymphomas were found after 15 days and about a 25% reduction in occurrence was found for each of the subsequent time points. Notably, a previous study by Battarchee showed that mice that received only a single 10 cGy dose did not exhibit an adaptive response.[10]

Another study shows a significant reduction in the number of thymic lymphomas induced in mice exposed to continuous irradiation. Ina *et al.* exposed mice to $2.88 \frac{\text{cGy}}{\text{day}}$ for up to 450 days. Some mice were irradiated with 7.2 Gy (fractionated into four doses) after 35 days while others were irradiated for up to 450 days without receiving a challenge dose. The mice irradiated for 35 days showed a 43% decrease in the number of lymphomas if the mice were kept irradiated after the challenge dose. Mice that were not continuously irradiated only showed a delay in the onset of lymphomas and no significant difference in the final number of lymphomas. The mice irradiated for 450 days received a total of 12.6 Gy, which is equal to the lethal dose with acute irradiation for those mice.[15] Not only was the frequency of thymic lymphomas reduced in the irradiated mice, but those mice showed no hair loss and weighed more than their control counterparts. Also, the mice irradiated up to 450 days had no thymic lymphomas. The study seemed to show that the adaptive response was dependent on the length and continuation of exposure, and that continuous low-dose irradiation increases the lifetime of mice.

Both of those studies used low doses, but those doses were still about 10,000 times (or four orders of magnitude) higher than background doses. Caratero *et al.* performed a study using $7-14 \frac{\text{cGy}}{\text{year}}$, which is 25 or 50 times higher than background. The study found that the irradiated population had a 20% increase in total lifespan. Thus far, low dose continuous irradiation appears to be promising for the induction and continuation of adaptive responses.

1.2 Objective

The goal of this project is to determine the effects of continuous low dose rates of ionizing radiation (x-rays and gamma rays) on cell proliferation in low and high oxygen conditions. Irradiators will be designed to deliver dose-rates to cells a few orders of magnitude above background to determine the long-term effects of radiation on cell proliferation. The irradiators will be placed in incubators set up to have either 5% (low) or 20% (high) ambient oxygen.

The source for the irradiators will be Americium-241 (Am^{241}). Am^{241} has a very long half life (over 400 years), removing the concern of having to replace the source due to decay.[16] The source is available in solid form as a flat and rectangular foil, facilitating handling and containment. Am^{241} decays by alpha emissions, which mostly lead to a 59.5 keV excited state of the daughter nuclide Neptunium-237 (Np^{237}). Two photons are emitted from Am^{241} : characteristic x-ray from Np^{237} and 59.5 keV gamma rays.[17]

Cells from two Chinese hamster ovaries (CHO) cell lines, CHO-K1 (wild type) and xrs-6 (Ku80 protein deficient), will be used in this experiment. The xrs-6 cell line is a mutant of the CHO-K1 cell line as it lacks Ku80 (X) an important gene in DNA double strand break (DSB) regulation.[18] xrs-6 cells, which are Ku-80 $-/-$, are more sensitive to radiation damage than CHO-K1.[19] Since the cells will be exposed to low doses and low dose rates, the use of both xrs-6 and CHO-K1 cells is important as radiation damage may not be observed in CHO-K1 cells.

Chapter 2

Methods

CHO-K1 cells were continuously exposed to the Am-241 foils for 7 weeks. The CyQUANT experiment was performed for the CHO-K1 cells on each of those weeks. The same experiment was setup for the xrs-6 cells. However, under the specified experimental procedures, it quickly became apparent that the irradiated xrs-6 cells did not survive to allow for a continuous experiment. The experiment was changed to irradiate xrs-6 cells for only one week, which repeated in triplicate.

2.1 Cell Culture

CHO-K1 cells and xrs-6 (Ku-80 knockout) cells used in this experiment were received from Larry H. Thompsons laboratory (Lawrence Livermore National Laboratory, CA). The cells were grown in HAM-F12 Minimum Essential Medium (Cellgro, Manassas, VA) and 10% fetal bovine serum (FBS, HyClone, Logan, UT). The cells were grown in incubators at 37°C, 5% CO₂, and either 5% or 25% O₂.

Each cell line was grown in a 12.5 cm² vented flask (Falcon, West Chester, PA). During each passage, the medium was first removed from the flask. The cells were rinsed with 5 mL of PBS, which was then aspirated out. The cells were given 0.5 mL of Trypsin (HyClone, Logan, UT) and incubated for 10 minutes at 37°C, 5% CO₂, and 25% O₂. To stop the effect of Trypsin, the cells were mixed in 5 mL of medium. The cell concentration was determined using a Coulter counter (Beckman,

Indianapolis, IN), mixing 0.100 mL of cell solution with 9.9 mL of distilled water. The cell concentration was determined by averaging three separate readings.

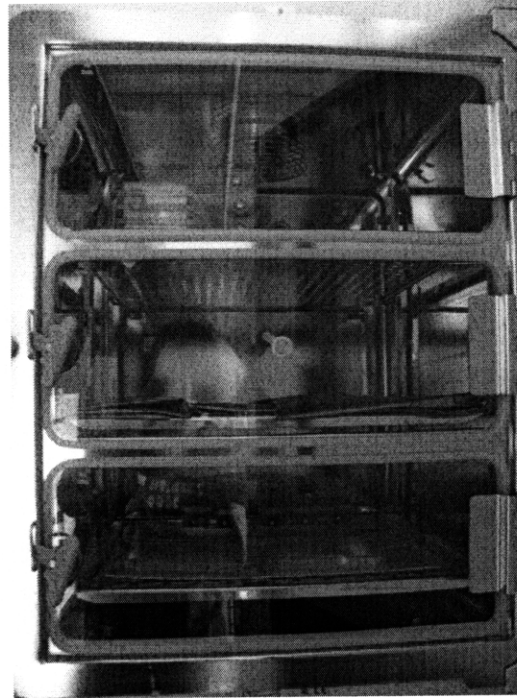
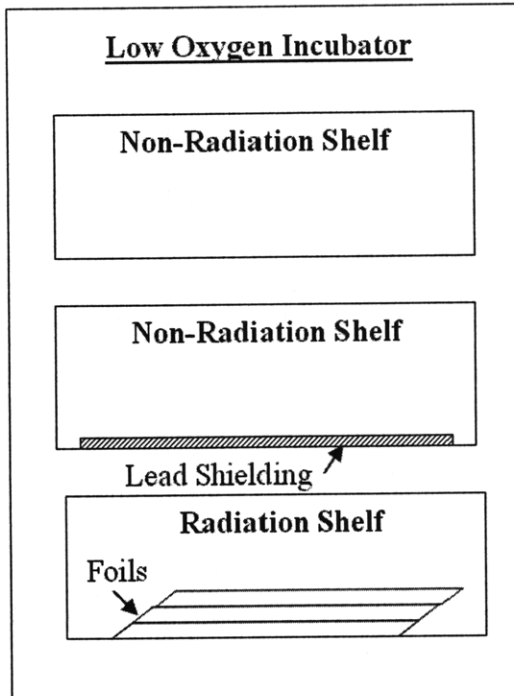
2.2 Am²⁴¹ Irradiator

The Am²⁴¹ foils used were purchased from NRD, LLC (Grand Island, NY). Americium powder was mixed with gold, compacted, then rolled into 14 in. x 3 in. foils. [20] The doses from the Am²⁴¹ were measured using a Landauer InLight Systems Microstar Reader (Glenwood, IL). Three InLight microdots were placed on a foil for 10 minutes. Each microdot was read 3 times before and after exposure, and the dose was determined as the average of the 3 readings with the associated error being the standard deviation of the 3 readings. The dose rate from the foils was found to be $1.76 \pm 0.02 \frac{cGy}{hr}$.

2.3 Incubator Set-up

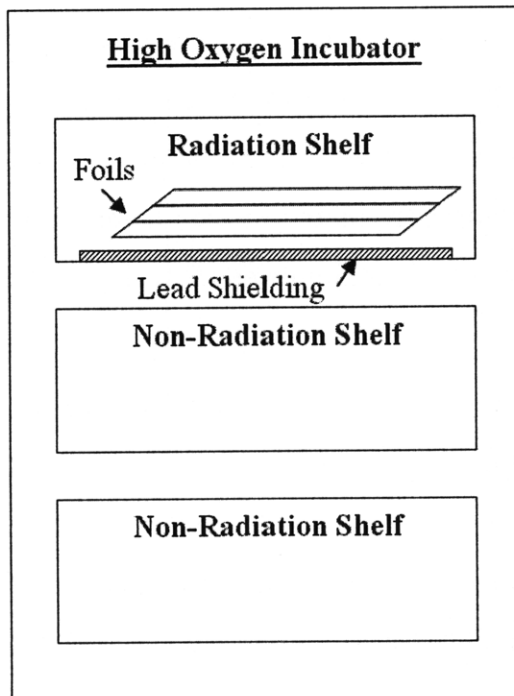
Two incubators were used to create four different environment conditions: high oxygen (20% O₂) without radiation (HNR), high oxygen with radiation (HR), low oxygen (5% O₂) without radiation (LNR), and low oxygen radiation (LR). One incubator was set-up with high oxygen, and the other with low oxygen. In each incubator, 3 Am²⁴¹ foils were placed on a shelf to create the continuous radiation environment, as depicted in Figure 2-1. The rest of the incubator was shielded from the radiation using 0.5" thick sheets of lead.

The low oxygen incubator was stacked on top of the high oxygen incubator. Lead was placed between the two incubators to prevent any radiation crossover. The dose rate in the shielded areas of the incubators was determined using Landauer microdots as described above. The dose rate in the shielded areas of the incubators was found to be $1 \pm 0.6 \times 10^{-3} \frac{cGy}{day}$ in the high oxygen incubator and $2 \pm 0.5 \times 10^{-3} \frac{cGy}{day}$ in the low oxygen incubator. The dose rates in the shielded areas are comparable to background dose rates ($0.7 \times 10^{-3} \frac{cGy}{day}$). [21]



(a) Schematic of low-oxygen incubator set-up.

(b) Picture of low-oxygen incubator set-up with flasks and plates.



(c) Schematic of high-oxygen incubator set-up.

(d) Picture of high-oxygen incubator set-up with plates and flasks.

Figure 2-1: Incubator setup for experiments in high and low O₂ conditions.

2.4 Cell Proliferation/CyQUANT experiment

The cells were plated in 96-well tissue culture plates (Falcon, West Chester, PA) and placed under four different conditions: HNR, HR, LNR, LR. For each condition, CHO-K1 and xrs-6 cells were plated on seven 96-well plates. In each plate, the cells were plated in 4 wells (for each cell line) at a density of 300 cells in 200 μ L of medium per well. The cells were left to settle in HNR condition for 6 hours before being placed in their respective condition. The cell dilution for each cell condition was plated in 4 wells and left to settle in HNR condition. After 6 hours, the medium was removed, and the plate was frozen at -80°C .

Cells were also plated in 12.5 cm² flasks (Falcon, West Chester, PA) to be used for the next experiment. For CHO-K1, 300 cells with 5 mL of medium were plated in each flask and placed under the corresponding condition in the incubator for one week. Three flasks were plated at the very beginning of the experiment, labeled Group 1 (G1), Group 2 (G2), and Group 3 (G3) cells respectively. G1 cells were used to prepare each week's CyQUANT experiment. G2 and G3 cells were maintained as backup in case G1 cells became infected. G2 and G3 cells were passage on different days: one day and two days after G1 passage respectively. For xrs-6, under HNR and LNR conditions, 300 cells with 5 mL of medium were plated in each flask; under HR and LR conditions, 300 and 5000 cells with 5 mL of medium were plated in each flask respectively.

Every day, 100 μ L of medium in each well was exchanged with 100 μ L of new medium. Also, one plate from each of the four conditions was frozen at -80°C every day (all medium was removed before freezing). After a week, all the plates were read using the CyQUANT Cell Proliferation Assay Kit (Invitrogen, Carlsbad, CA). A solution of distilled water, 20-fold dilution of cell-lysis buffer, and 400 fold dilution of green fluorescent CyQUANT GR dye was prepared and placed in each well for five minutes before reading. The plates were kept in the dark under aluminum foils during that time.

The CyQUANT cell proliferation assay works by having the cell-lysis buffer break

up the cells and then the green fluorescence dye attach to cellular nucleic acids. [22] The intensity of the fluorescence is then indicative of the number of cells in each reading.

The plates were read using a Spectra Gemini Spectrofluorometer (Molecular Devices, Sunnyvale, CA) and the SOFTmax Pro software. The software was setup to perform 6 readings per well to provide the intensity in each well. The intensity for each day, cell line, and condition was determined by averaging the readings from the corresponding four wells in each plate.

2.5 Statistical Analysis

Data reported were the average of three technical repeats within each experiment. They were represented as $\mu \pm \sigma$ with a 95% confidence level. All confidence levels were determined using the Students t-test.[23]

Chapter 3

Results

The goal of this experiment was to determine if CHO-K1 and xrs-6 cells would change their proliferation rate after 7 weeks of continuous irradiation. The results first compare the relative growth in number of CHO-K1 cells (in high oxygen conditions) over each week to that of the other weeks. The results also compare the number of cells in CHO-K1 flasks at the end of each week. Three experiments, run at different times, during which xrs-6 cells (in high oxygen conditions) are exposed to one week of radiation are compared. Finally, the results look at any differences between the growth of CHO-K1 and xrs-6 cells when placed in high oxygen vs. low oxygen conditions.

3.1 Standard Curve

A reference standard curve, shown in Figure 3-1 was created to relate the fluorescence intensity to the number of cells in the CyQUANT assay. The reliable threshold for the number of cells determined by the CyQUANT assay is about 50,000 cells.[22, 24] A fluorescence intensity of about 800 was found to represent 50,000 cells in a well, as shown in Figure 3-1. In analyzing the data, it was observed that fluorescence in each well reached an intensity of 800 around 5 days into the week and leveled off afterwards. However, it was known that the cells kept growing after day 5. Therefore, the following results only show cell proliferation data up to day 5 for each experiment.

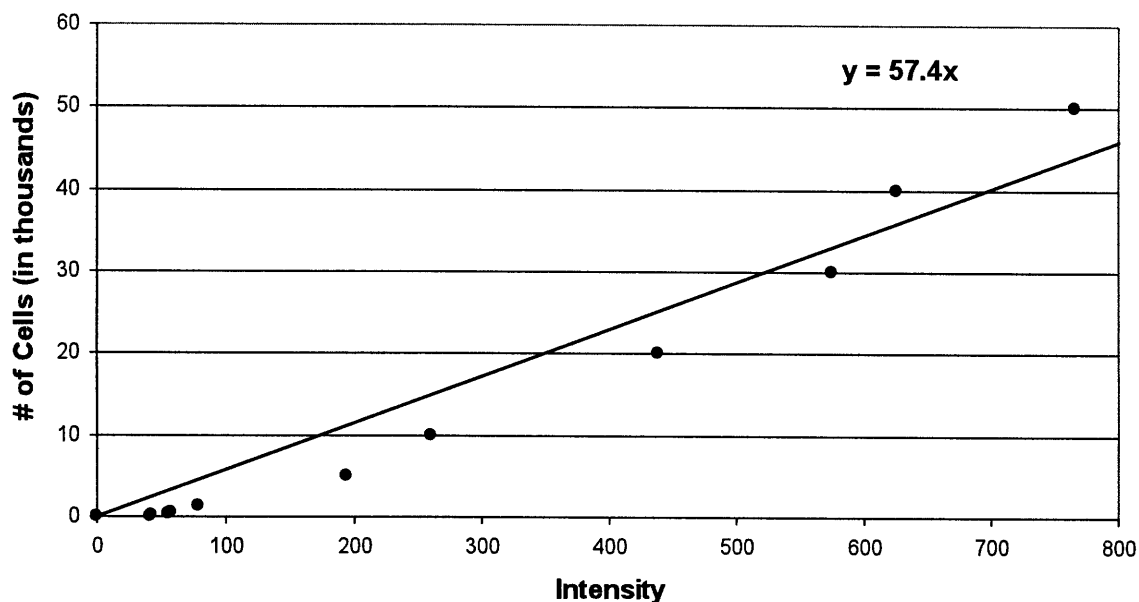


Figure 3-1: Standard Curve for CHO-K1

3.2 CHO-K1 Continuous Irradiation

CHO-K1 cells were continuously irradiated for 7 weeks. The data were analyzed by comparing the relative intensity found in each plate in a week. For each week, the intensity found on Day 1 was used to normalize all subsequent intensity.

Figures 3-2 and 3-3 show the comparison of the daily relative intensity of control CHO-K1 cells for weeks 2 to 7. A few data sets were omitted. Week 1 data will be treated subsequently. Week 4 data were unreliable as the 96-well plates were mixed up and placed on the wrong conditions. For week 6, no data point is available on Day 5 as signs of infections began.

The relative intensity of the comparison growth curve increases dramatically during day 5. A study by Daniotti *et al.* observed the proliferation of CHO-K1 by counting the number of cells each day using a Neubauer chamber. The number of cells recorded in the Daniotti *et al.* study was an order of magnitude greater than the ones in this experiment. The number of cells in each well during day 5 had likely reached fluorescence saturation, thus explaining the lower relative intensity observed on Day 5 for weeks 2 to 7 when compared to the reported CHO-K1 growth.

The control CHO-K1 cells were expected to grow as observed in other studies.

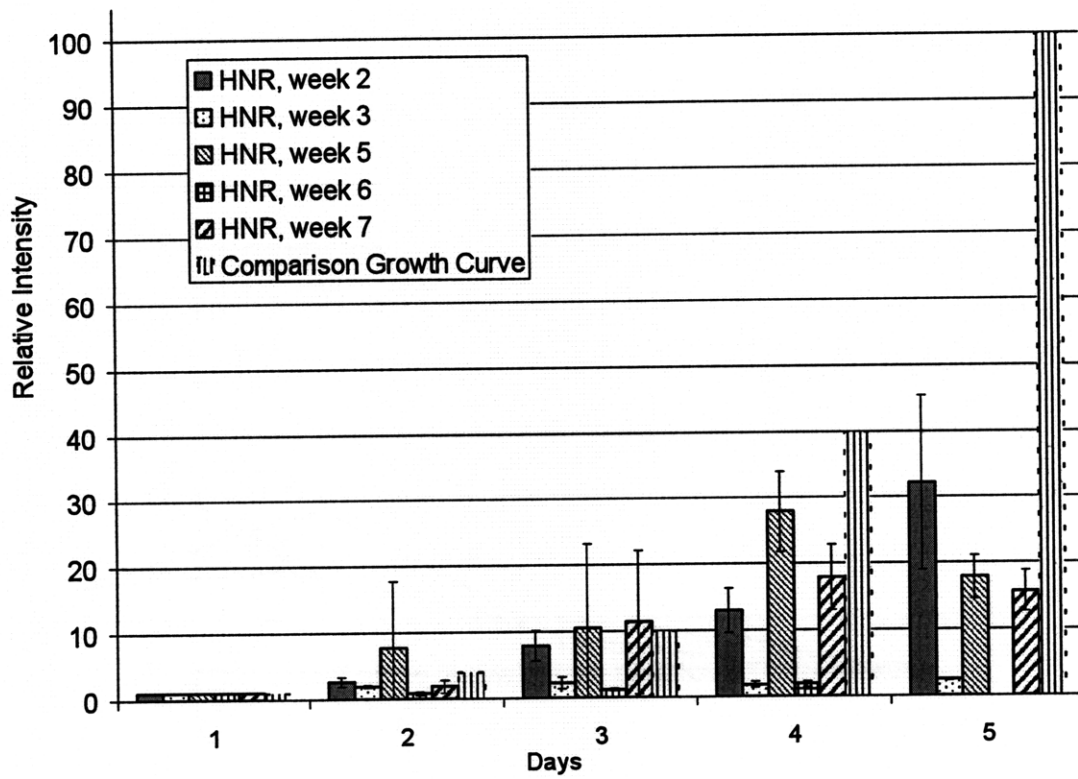


Figure 3-2: Control CHO-K1 comparison of relative intensity (relative number of cells) in daily plates for weeks 2 to 7. The comparison growth curve was generated from the results by J.L. Daniotti *et al.*

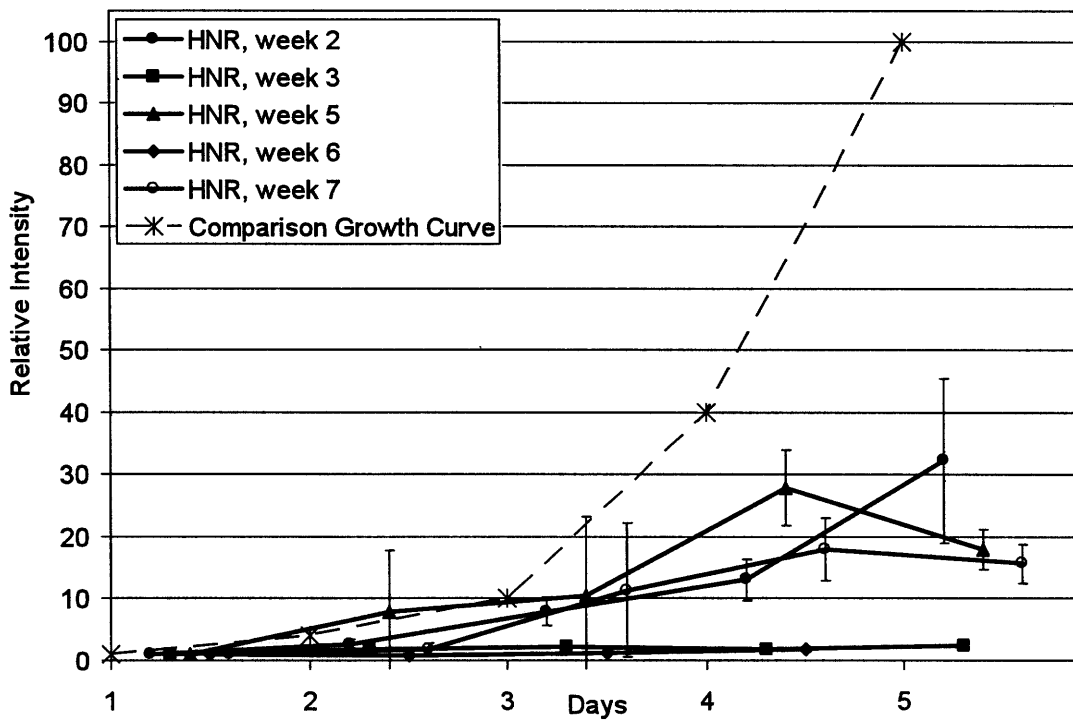


Figure 3-3: Control CHO-K1 comparison of relative intensity (relative number of cells) in daily plates for weeks 2 to 7. Data points are staggered to distinguish the error bars. The comparison growth curve was generated from the results by J.L. Daniotti *et al.*

Week 2, week 5, and week 7 followed the comparison growth curve until Day 5. However, it appears that no cells grew during week 3 and week 6 experiments.

Figure 3-4 shows the comparison of the daily relative intensity of irradiated CHO-K1 cells for weeks 2 to 7.

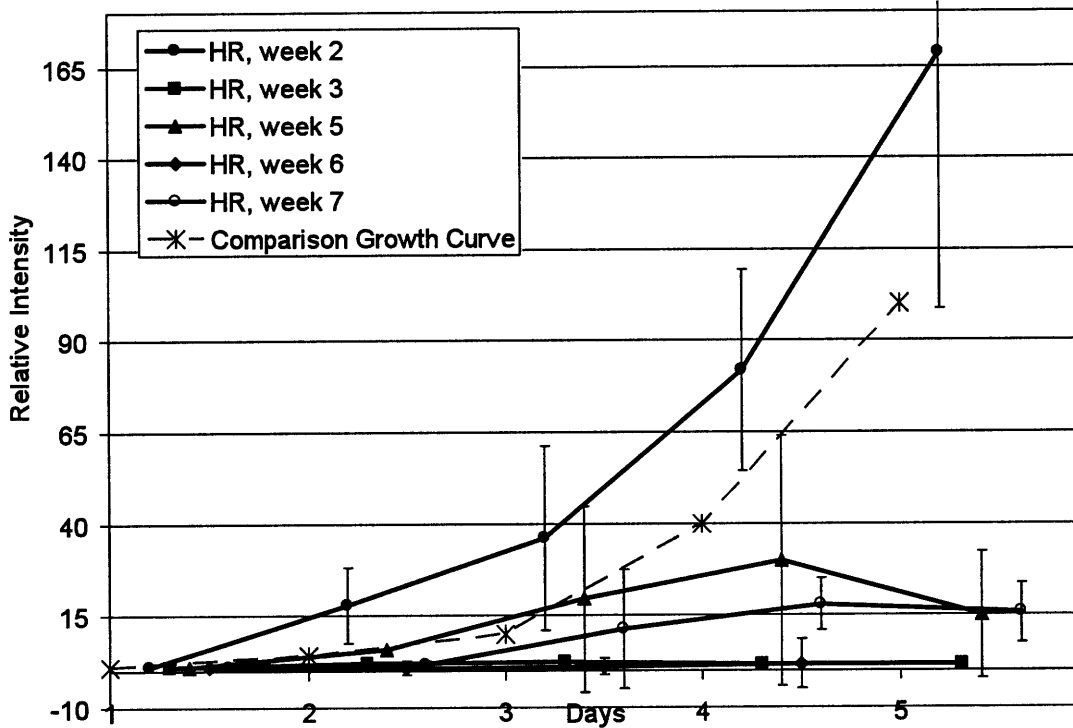


Figure 3-4: Irradiated CHO-K1 comparison of relative intensity (relative number of cells) in daily plates for weeks 2 to 7. Data points are staggered to distinguish the error bars. The comparison growth curve was generated from the results by J.L. Daniotti *et al.* for non-irradiated cells.

Week 2 irradiated CHO-K1 cells appeared to be close to the comparison growth curve. Week 5 and week 7 irradiated CHO-K1 cells grew until day 4, after which there appears to be a dip in intensity. Once again, week 3 and week 6 cells showed no growth.

Figures 3-5 and 3-6 compare the relative intensity of control and irradiated CHO-K1 cells for weeks 2,5, and 7. Week 3 and week 6 data were omitted as the results showed that there was no cell growth in the plates suggesting that not enough cells were plated at the beginning of the experiment.

Only week 2 irradiated CHO-K1 cells showed a statistically different growth com-

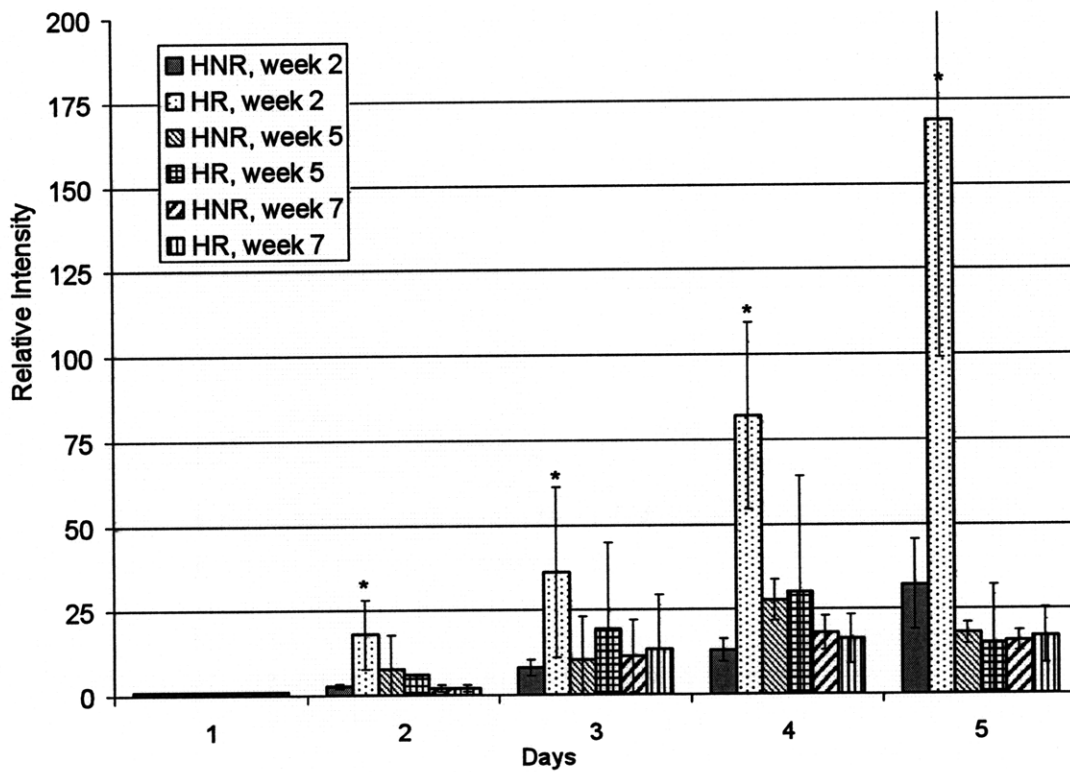


Figure 3-5: Control and Irradiated CHO-K1 comparison of relative intensity (relative number of cells) in daily plates for weeks 2, 5, and 7. An asterisk (*) indicates $p \leq 0.05$. Statistical significance is determined by comparison with HNR control.

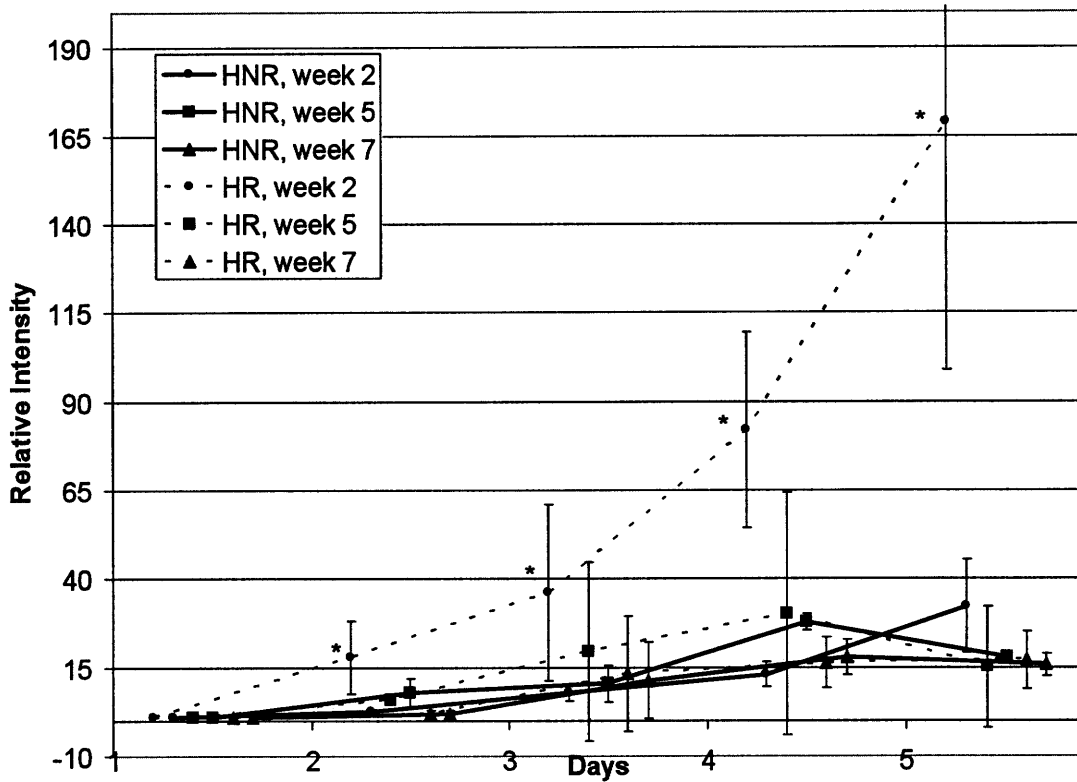


Figure 3-6: Control and Irradiated CHO-K1 comparison of relative intensity (relative number of cells) in daily plates for weeks 2, 5, and 7. Data points are staggered to distinguish the error bars. An asterisk (*) indicates $p \leq 0.05$. Statistical significance is determined by comparison with HNR control at corresponding week.

pared to the control cells. For week 5 and week 7, it appeared that both irradiated and control CHO-K1 cells had similar growth patterns.

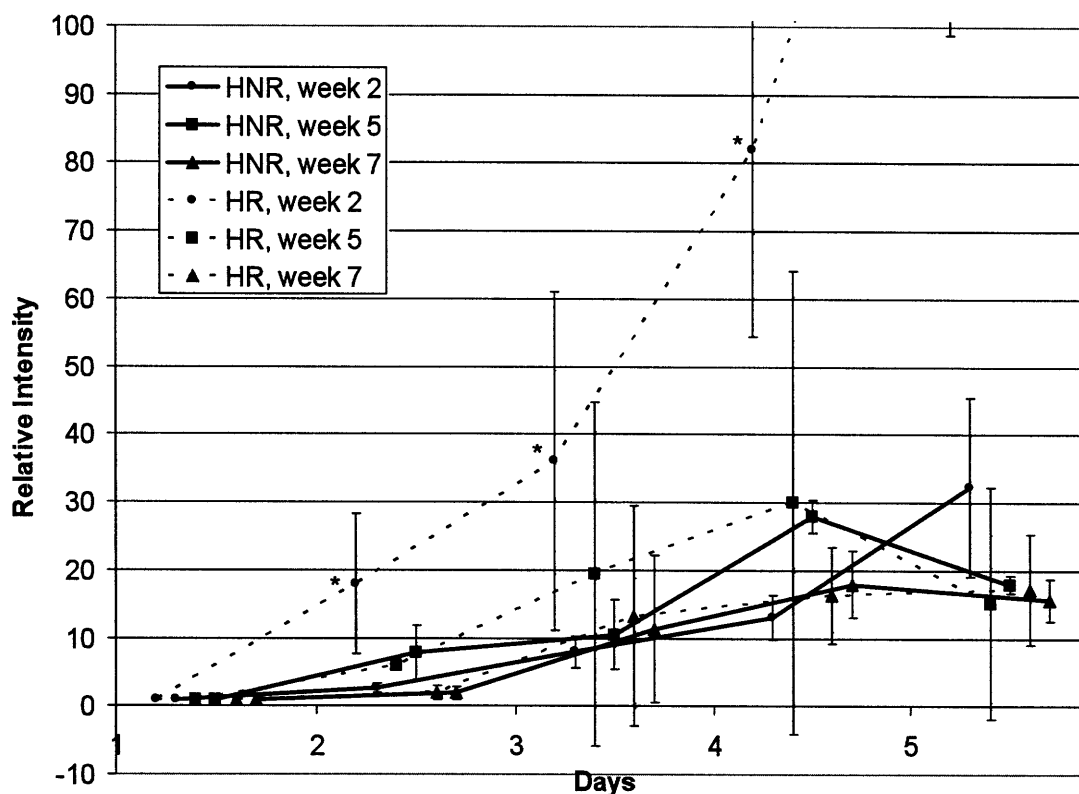


Figure 3-7: Expanded view on control and irradiated CHO-K1 comparison of relative intensity (relative number of cells) in daily plates for weeks 2, 5, and 7. Data points are staggered to distinguish the error bars. An asterisk (*) indicates $p \leq 0.05$. Statistical significance is determined by comparison with HNR control.

Every week, the number of cells in each group 1 flask was counted using a Coulter Counter to setup for the following weeks experiment. Figure 3-8 shows the number of cells in each CHO-K1 flask under the various conditions (HR, HNR, LR, LNR) after each week of continuous irradiation.

The CHO-K1 growth in plates showed no difference between the irradiated and control cells. However, the total cell number collected from the flasks at the end of every week seems to indicate that non-irradiated CHO-K1 cells proliferated faster. There is a dip in the number of control CHO-K1 cells after 6 weeks of exposure because the flask used then was a group 2 flask (group 1 flask for that particular week was unusable), which had only been passaged 5 days before counting instead of

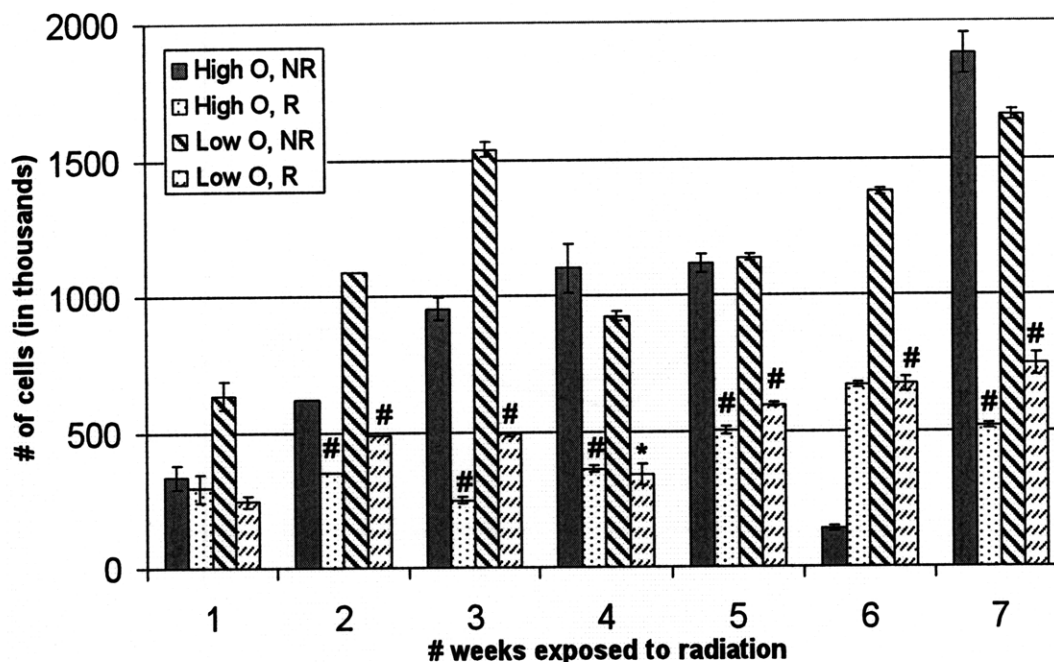


Figure 3-8: Control and irradiated CHO-K1 comparison of number of cells in each flask at the end of every week for the four conditions: HNR, HR, LNR, LR. An asterisk (*) indicates $p \leq 0.05$. A pound (#) indicates $p \leq 0.01$. Statistical significance is determined by comparison with non-irradiated control.

7 days.

Figure 3-9 compares the relative number of irradiated cells to control cells. The same number of cells was originally plated for all conditions; however the irradiated cells showed lower rates of proliferation. After seven weeks of continuous irradiation, the CHO-K1 cells in the irradiated flasks showed a factor of 2 decrease in cell growth when compared to the non-irradiated flasks.

3.3 xrs-6 after 1 Week of Irradiation

xrs-6 cells were exposed to radiation for one week under high oxygen condition. The experiment was repeated three times. Of the three trials, only one set showed a statistically significant difference between the growth of irradiated and control xrs-6 cells, as shown in Figures 3-10 and 3-11.

A 7-week experiment during which the xrs-6 cells were continuously irradiated could not be accomplished. Every flask plated with 300 xrs-6 cells at the beginning of

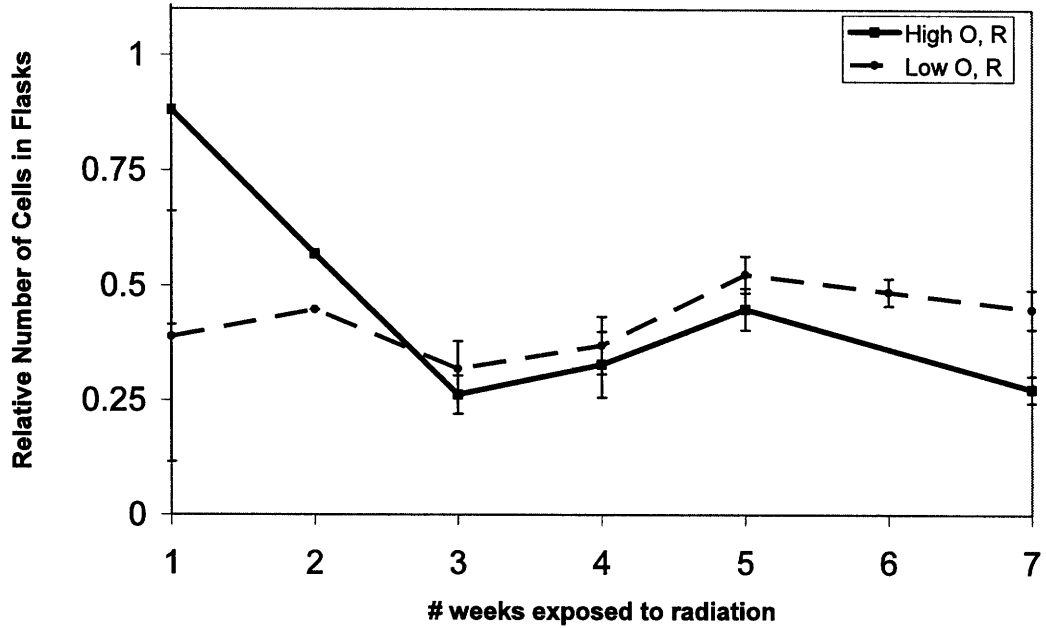


Figure 3-9: Relative number of cells in the irradiated flasks compared to control flasks. HNR and LNR curves normalized to 1. Day 6 data point for HNR was omitted as it was derived from a different flask group.

the experiment and placed on the Am^{241} foils showed little, if any, cell proliferation. The cell count on the Coulter Counter was indistinguishable from background counts (i.e. counts recorded by the Coulter Counter without any cells in the cuvette). However, control flasks plated with 300 xrs-6 cells did grow. Irradiated flasks plated with 5000 xrs-6 cells (instead of 300) also managed to grow. Trial shows the differences in cell counts after Trial 3.

Table 3.1: Comparison of cell density in control and irradiated xrs-6 flasks

<i>Condition</i>	<i>Cell Density</i> $\frac{\text{cells}}{\text{mL}}$
Background	21 ± 10
High O_2 , Control	832 ± 18
High O_2 , γ	9 ± 5
Low O_2 , Control	1313 ± 165
Low O_2 , γ	20 ± 9

The cell counts observed for the irradiated cells were much lower than the counts for the control cells. In the high oxygen conditions, the cell count for control cells was 100 times higher than that of irradiated cells. In the low oxygen conditions,

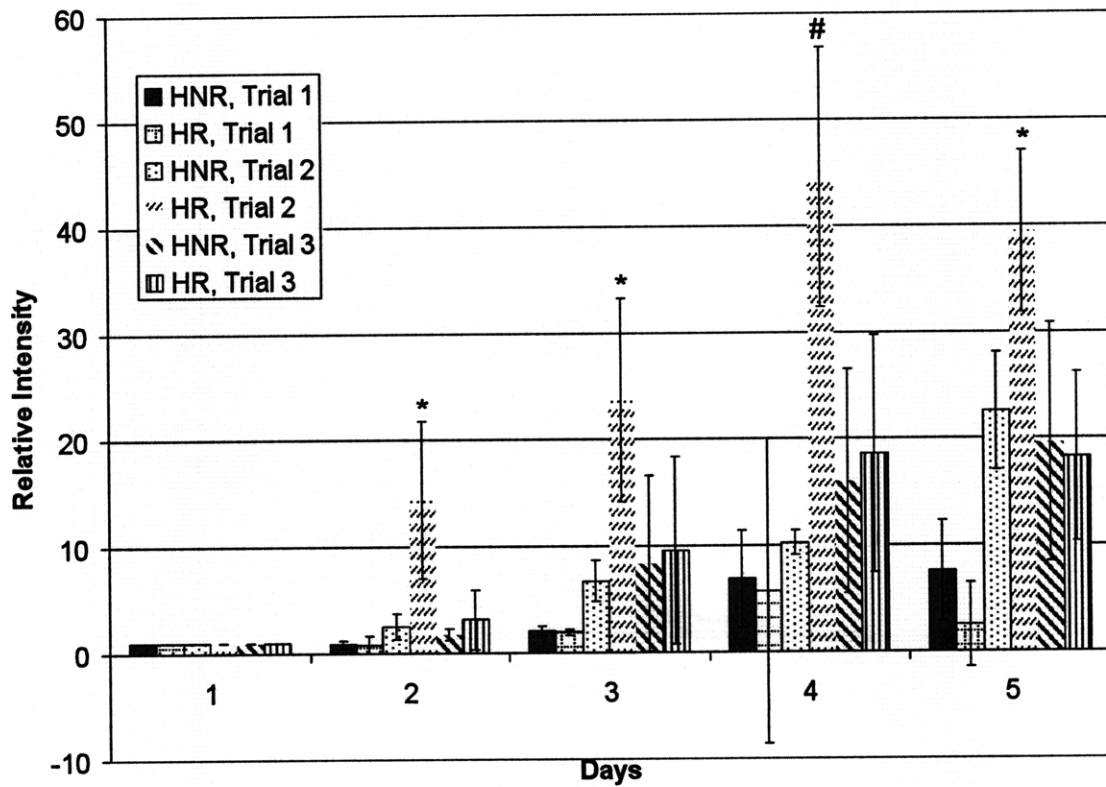


Figure 3-10: Control and irradiated xrs-6 comparison of relative intensity (relative number of cells) in daily plates for week 1. An asterisk (*) indicates $p \leq 0.05$. A pound (#) indicates $p \leq 0.01$. Statistical significance is determined by comparison with HNR control for each corresponding trial.

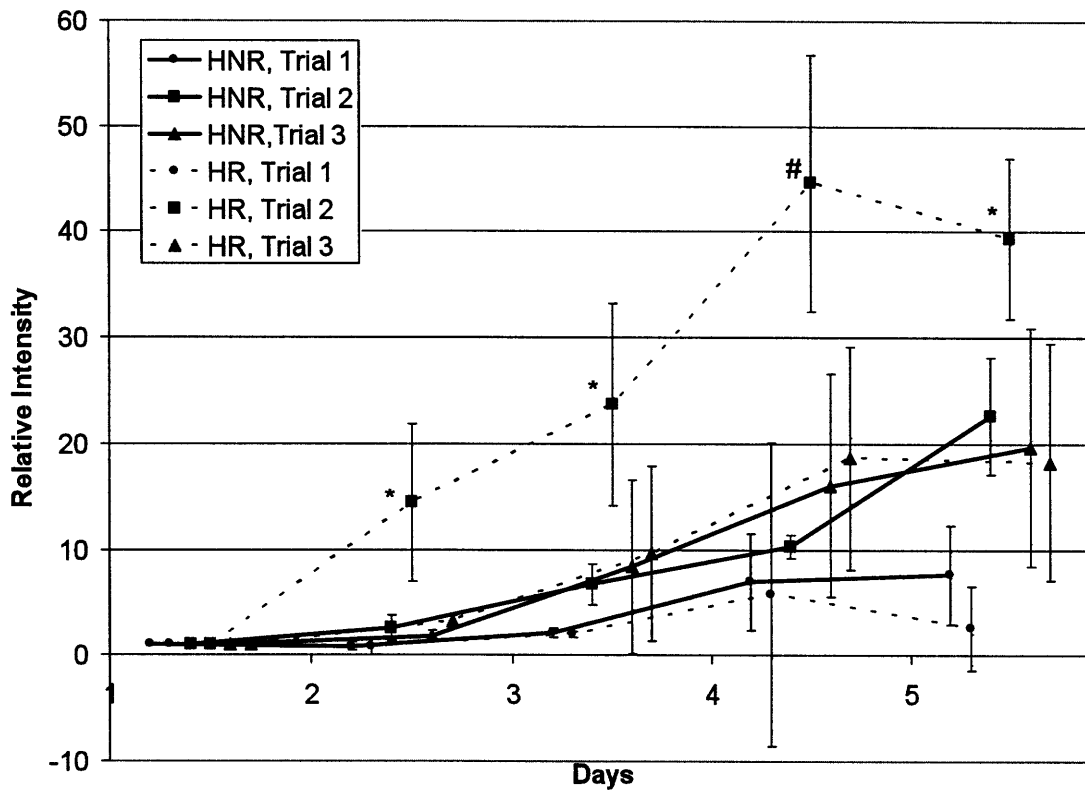


Figure 3-11: Control and irradiated xrs-6 comparison of relative intensity (relative number of cells) in daily plates for week 1. An asterisk (*) indicates $p \leq 0.05$. A pound (#) indicates $p \leq 0.01$. Statistical significance is determined by comparison with HNR control for each corresponding trial.

the control cell count was 50 times higher than irradiated count.

3.4 CHO-K1 after 1 Week of Irradiation

CHO-K1 cells were exposed to radiation from the Am²⁴¹ foils for one week in the high-oxygen incubator. The experiment was performed three times. For each experiment, there was no statistical difference between the relative intensity of CHO-K1 cells exposed to radiation compared to that of the non-irradiated cells, as shown in Figures 3-12 and 3-13.

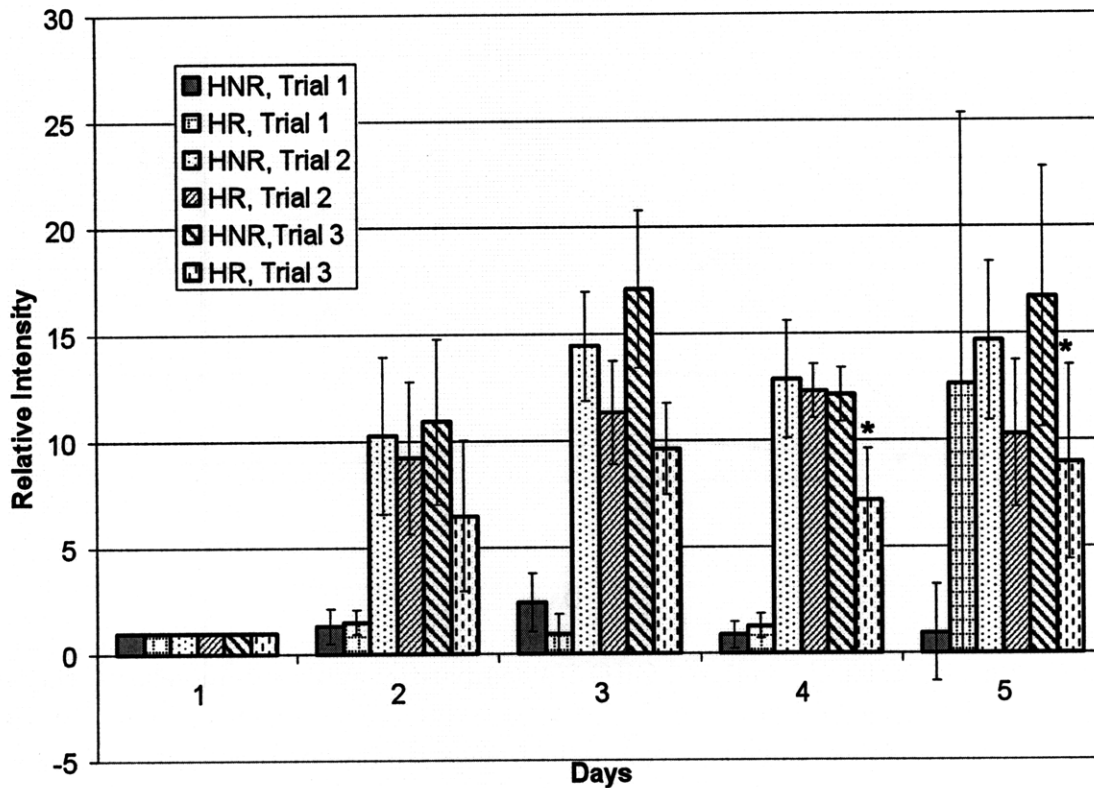


Figure 3-12: Control and irradiated CHO-K1 comparison of relative intensity (relative number of cells) in daily plates for week 1. An asterisk (*) indicates $p \leq 0.05$. Statistical significance is determined by comparison with HNR control for each respective week.

As shown in Figures 3-12 and 3-13, Trial 1 was significantly different from Trial 2 and Trial 3. Trial 2 and Trial 3 were conducted during the same week, which could account for their similarity. Trial 1 was conducted at the very beginning of

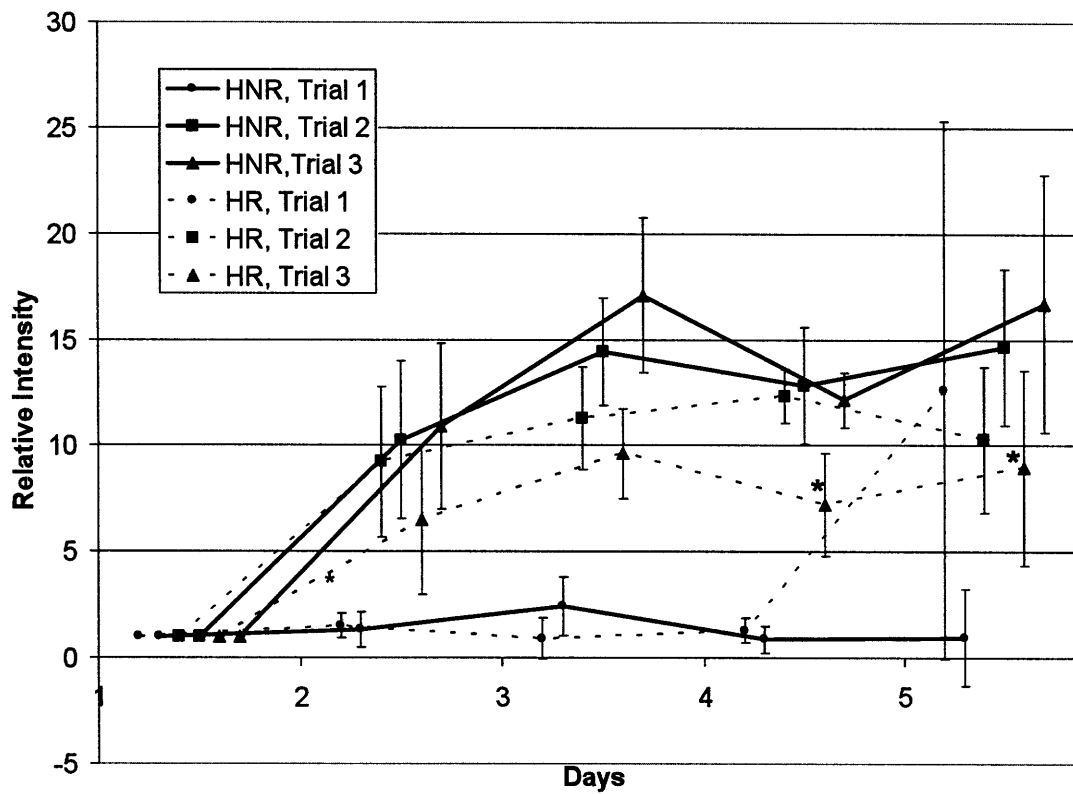


Figure 3-13: Control and irradiated CHO-K1 comparison of relative intensity (relative number of cells) in daily plates for week 1. An asterisk (*) indicates $p \leq 0.05$. Statistical significance is determined by comparison with HNR control for each respective week.

the experiment, during which plating technique might not have been as accurate as during the following experiments. As the CHO-K1, week 1 results were inconclusive, they were treated separate from the continuous CHO-K1 experiment.

3.5 High vs. Low Oxygen

Differences in cell proliferation for both CHO-K1 and xrs-6 cells were not observed between high and low oxygen conditions. Looking at CHO-K1 cells that are going through two weeks of irradiation, the only difference appears for the cells in HR condition, as seen in Figures 3-14 and 3-15.

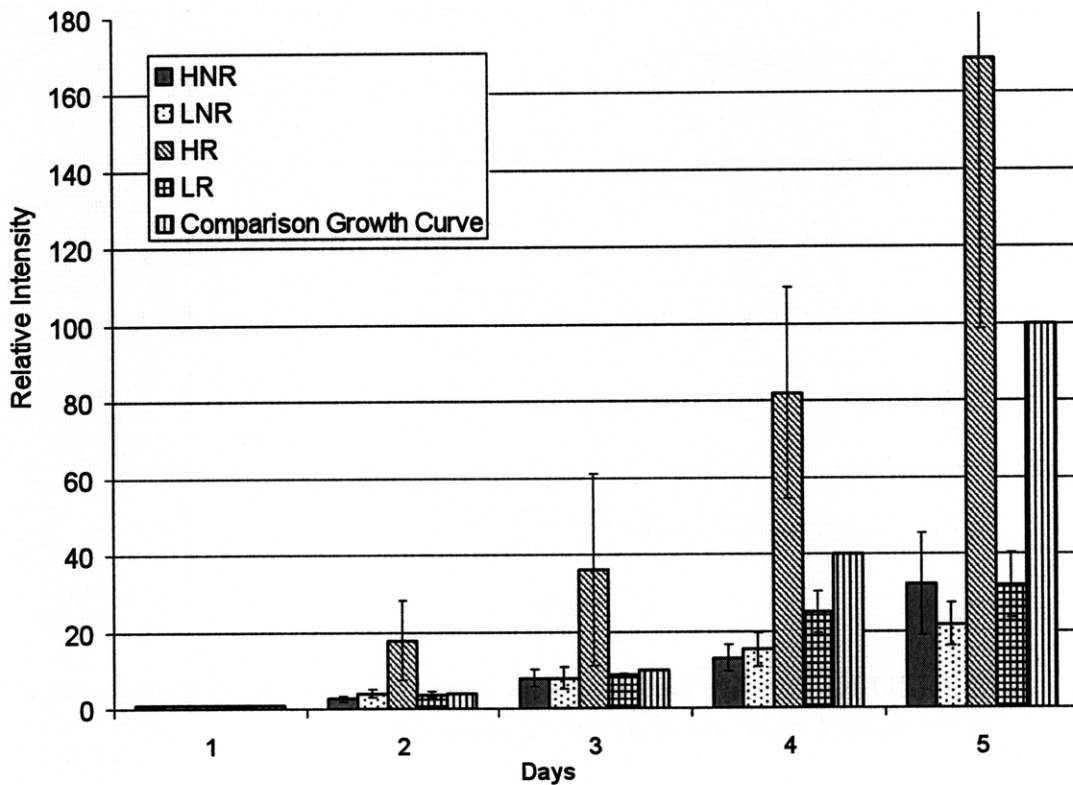


Figure 3-14: CHO-K1 comparison of relative intensity (relative number of cells) in daily plates for week 2 in high and low oxygen conditions.

However, this phenomenon is not seen again in the rest of the experiments. No other significant differences between low and high oxygen conditions were observed for CHO-K1 cells.

xrs-6 cells also showed no significant differences between low and high oxygen

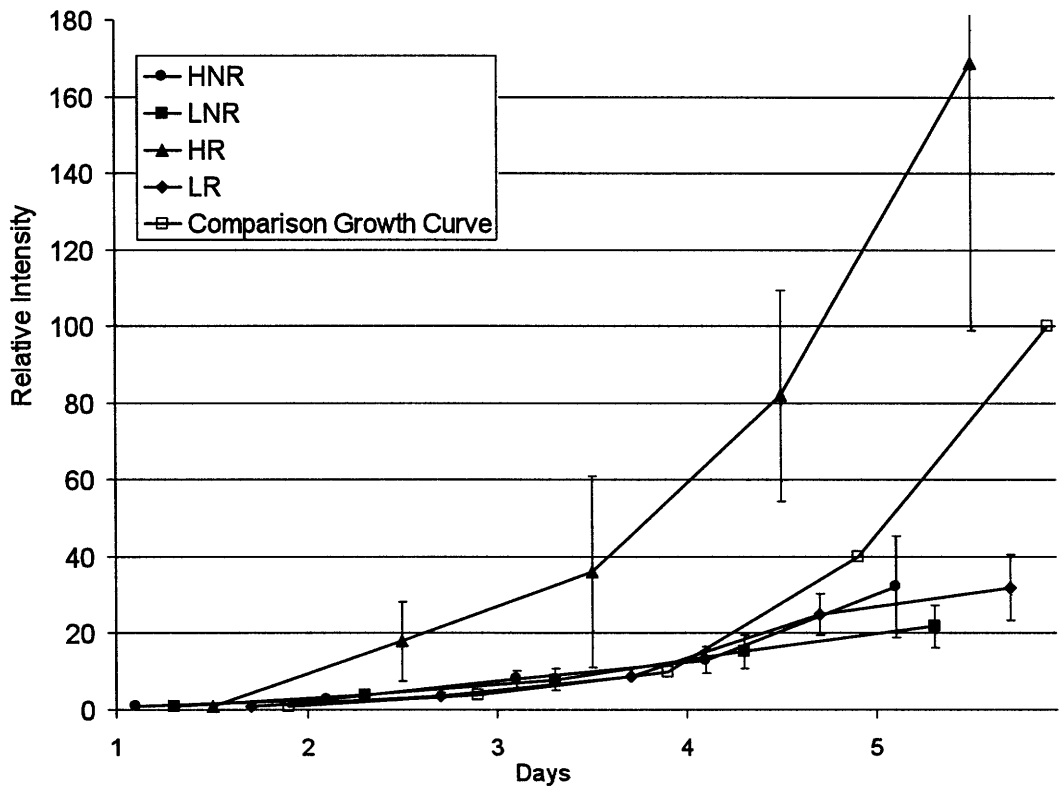


Figure 3-15: CHO-K1 comparison of relative intensity (relative number of cells) in daily plates for week 2 in high and low oxygen conditions.

conditions. The comparison in Figures 3-16 and 3-17 used the average of the relative intensity from the three xrs-6 (week 1) trials.

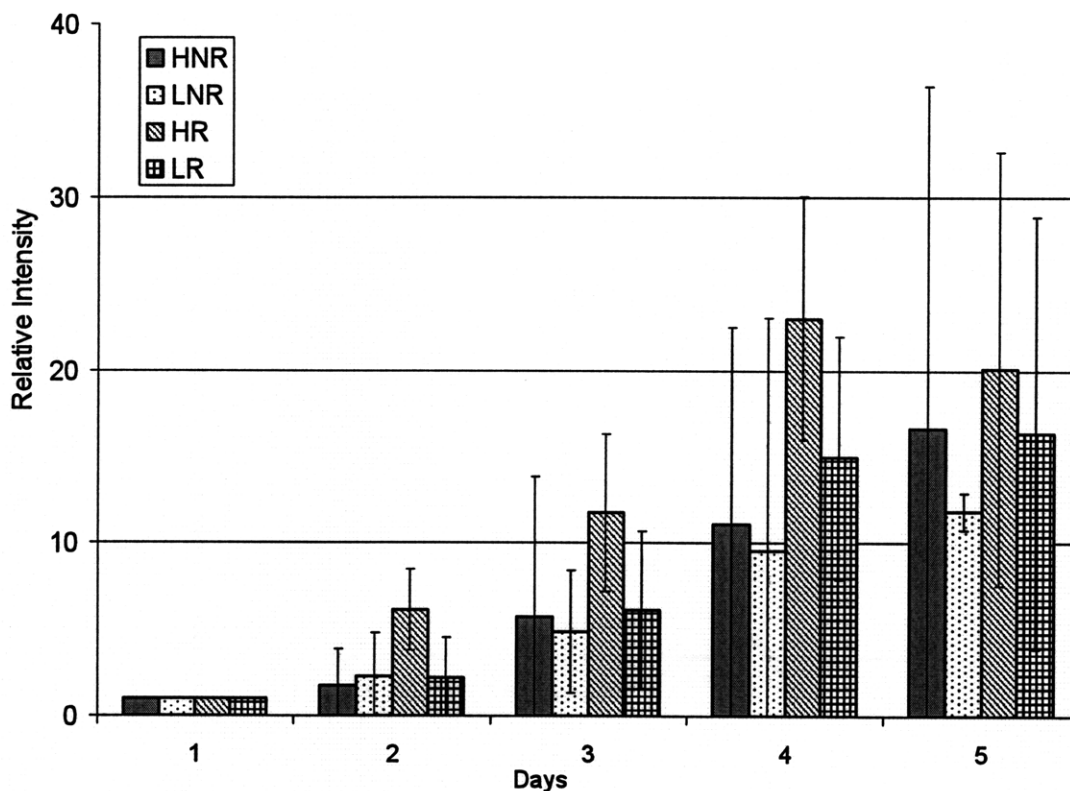


Figure 3-16: xrs-6 comparison of relative intensity (relative number of cells) in high and low oxygen conditions. The relative intensity was calculated as the average of the three xrs-6 trials.

Some of the standard deviations in Figures 3-16 and 3-17 are considerable due to the low values from Trial 1. However, the overarching message is that oxygen levels did appear to create significant differences in cell proliferation in both CHO-K1 and xrs-6 cells.

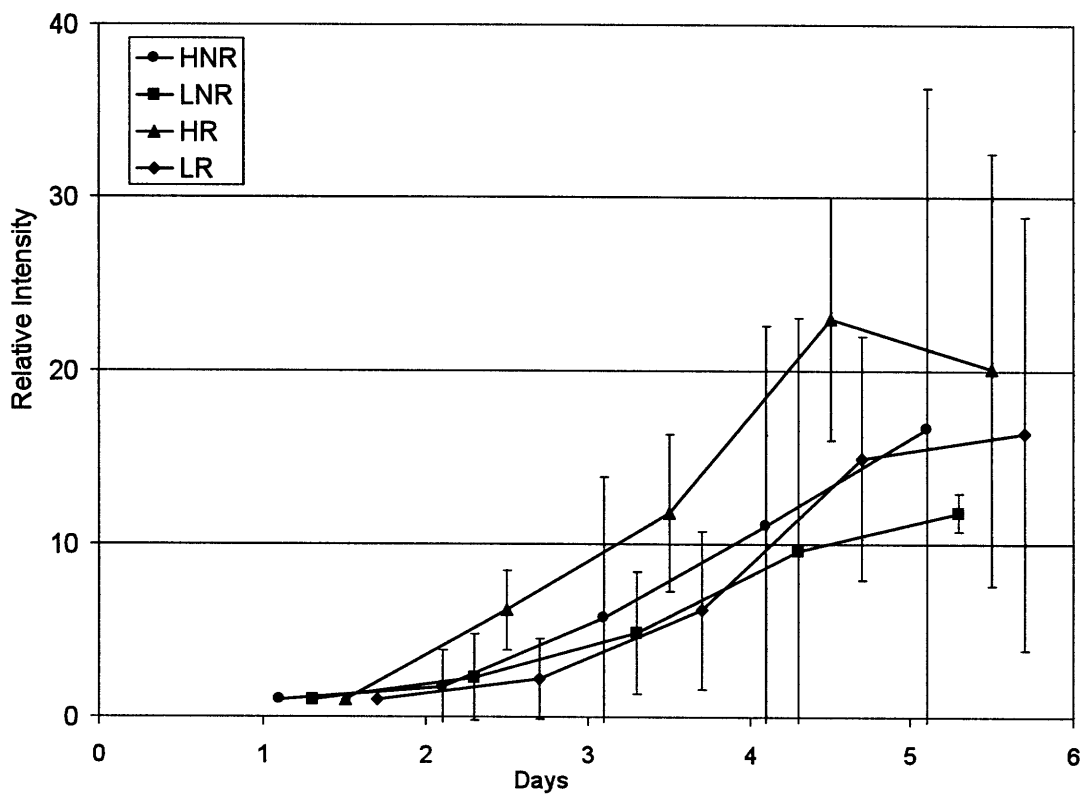


Figure 3-17: xrs-6 comparison of relative intensity (relative number of cells) in high and low oxygen conditions. The relative intensity was calculated as the average of the three xrs-6 trials.

Chapter 4

Discussion

4.1 CHO-K1 Continuous Irradiation

CHO-K1 cells were continuously irradiated on the Am²⁴¹ foils. After 7 weeks, the CHO-K1 cells did not show signs of an increase or decrease in proliferation rate when compared to growth of the control cells. It is possible that the length of this experiment was too short to observe a response in the CHO-K1 proliferation rate. A study by Satta *et al.* in which cells were placed under continuous radiation at lower than background dose rates did not induce a change in proliferation after 3 months. However, after 9 months, a statistically significant increase in proliferation was observed for the cells that had been exposed to the lower radiation dose rate.[25]

Changes in cell proliferation are expected to come with a slowing or stopping of some or all the cell cycle phases.[26] Cell proliferation changes during irradiation has been attributed to perturbations in the progress of the G2 phase of mitosis.[27] Since no significant changes in the CHO-K1 growth rate were observed, it appears that the cells are well suited to mitigate the damage through the cell cycle phases. Another hypothesis is that the cells that are still growing by the 7th week of irradiation are naturally selected cells and hence are inherently better able to handle the radiation damage.

The cells survived a considerable dose of about 20 Gy over the 7 week period. A clonogenic assay on CHO-K1 and xrs-6 cells showed that an acute dose of 8 Gy and

3 Gy respectively is enough to reproductively kill over 90% of the population.

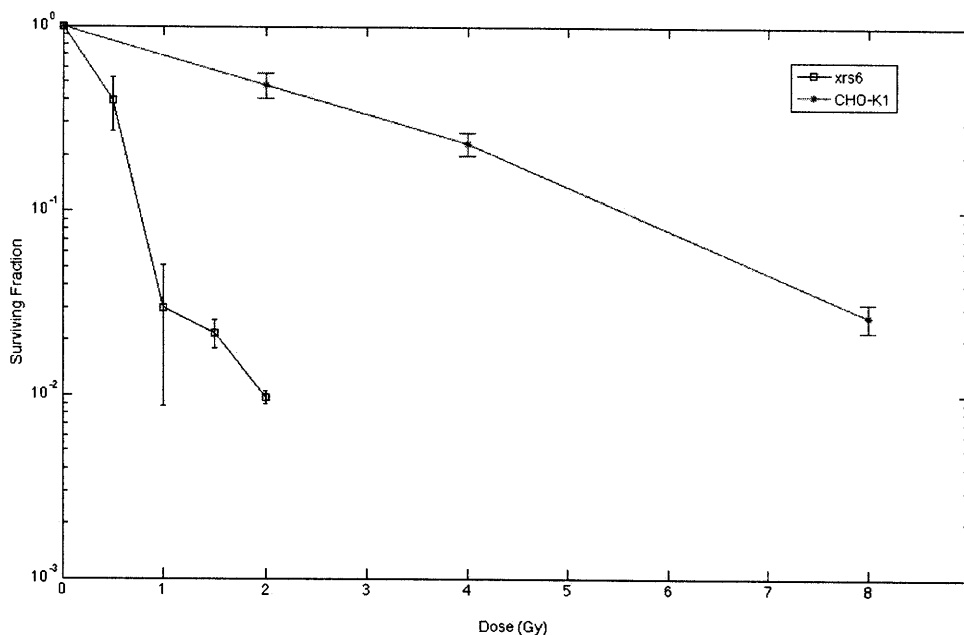


Figure 4-1: CHO-K1 and xrs-6 survival curve, courtesy of D. Chambers and L. Torres (unpublished). Dose delivered acutely using a 250 kVp x-ray spectrum.

As shown in this work, the CHO-K1 cells are able to survive a large total dose when delivered at low dose rates that would otherwise be lethal if delivered acutely. The CHO-K1 cells received a total dose of over 20 Gy at a rate of $1.73 \frac{cGy}{hr}$ over the 7-week period, yet no change in proliferation rate was observed. Other studies have shown that cells and mice are able to handle continuous low doses of radiation and eventually receive a large total dose that would be lethal if received acutely. As mentioned in Section 1.2.2, the work by Ina *et al.* irradiated mice for 250 days, delivering a dose of 12.6 Gy. E. Elmore *et al.* irradiated HeLa skin fibroblast human hybrid cells with $4 \frac{mGy}{day}$ for 88 days. When the irradiated cells were challenged with a high dose, those cells had a reduced number of neoplastic transformations as compared to the control cells.

Errors in the experimental setup and methodology could also have affected the results. The variation in the number of cells initially plated for each experiment introduces errors, which were somewhat mitigated by looking at relative intensity instead of absolute cell numbers. For week 3 and week 6 experiments, it seems that

the dilution for the CHO-K1 cells were too low and thus did not allow any cells to grow in the plates.

For the CyQUANT assay, a solution was prepared weekly to read the data from each experiment. Small variations in the solution, such as the quality of mixing the solution or length of wait time before use, could have affected the fluorescence results. For example, some wells could have received a higher concentration of fluorescent dye, while others received less.

The medium in each plate was changed every day, which increased the chances of infections. Notably, during week 5 experiment, fungus was observed in the plates after day 5. It is also possible that cells switch between wells in each plate via transfer of medium, introducing more noise in the results.

4.2 xrs6 after 1 Week of Irradiation

xrs-6 cells are known to be more sensitive to radiation as they lack the Ku80 protein, a key player in double strand break repair. [18, 19] The xrs-6 cells were expected to demonstrate a response when continuously irradiated on the Am²⁴¹ foils. The irradiated cells plated in the 96-well plates did not appear to grow much less than their control counterparts. However, the key observation was that the irradiated cells plated in the flasks did not grow well compared to the non-irradiated cells in the flasks. This phenomenon was also observed in with the CHO-K1 cells, though at a lesser extent.

The difference in cell growth between the plates and the flasks is most likely attributed to cell density on the plating area. The area of the wells in the 96-well plates is significantly smaller than that of the flask. Hence, the cell density in the wells is much higher. Cells are known to release growth factors, inducing more growth among the other cells.[28, 29] This phenomenon is also observed in the CHO-K1 and xrs-6 as growth appears to be faster at later days as the cells become more confluent. For xrs-6, not enough cells in the flasks appeared to have survived the first few days on the Am²⁴¹ foils to continue to grow. The same number of xrs-6 cells likely died

in the wells; however, enough of them survived in close proximity of each other to be able to grow. When the number of xrs-6 cells plated in the flasks was increased from 300 to 5000 cells, the cells were able to grow and populate the flasks.

4.3 CHO-K1 after 1 Week of Irradiation

The results for the CHO-K1 radiation for the first week were very puzzling. The growth of the irradiated cells was expected to follow that of the control cells within each trial. However, the three separate trials did not appear to concur with one another as to how much growth occurred. The differences in growth were mostly attributed to errors in plating and differences in the quality of the CyQUANT solution.

4.4 Low vs. High Oxygen Content

Oxygen tension has been found to regulate cell proliferation. Human dermal fibroblasts were found to proliferate better under low oxygen conditions [30] , while mice tumor cell growth decreased with lower oxygen concentration.[31] Since oxygen can become a source of DNA damage as a free radical, it was expected that cells would grow better when irradiated at low oxygen and at high oxygen.[32] However, neither CHO-K1 nor xrs-6 cells showed any significant difference in growth when exposed to low and high oxygen conditions.

The difficulty in running experiments at low oxygen concentration is that the cells are inevitably exposed to high oxygen conditions when they are passaged or getting ready for the next experiment. This repeated spike in oxygen level may have distorted the results of the low oxygen conditions.

Chapter 5

Conclusion

There is much to discover on the effect of low doses and low dose rates of ionizing radiation. Though there was no significant change in cell proliferation for the CHO-K1 cells, the cells were able to handle a large cumulative dose of 20 Gy. The xrs-6 cells were not able to grow when plated at a low cell density but did manage to thrive once a threshold density was achieved. There are many questions to be answered from the results in this experiment: what growth factors are at play? How is the genetic makeup of the cells changing with irradiation? What adaptive response is being developed, if any?

Further work should be pursued to determine if the cells developed an adaptive response such as being more apt to repair DNA damage. Clonogenic assays should be performed on the continuously irradiated cells to determine the onset and duration of an adaptive response. Studies have shown that the adaptive response takes some time to appear after the conditioning dose is induced and eventually disappears.[5, 6] The CHO-K1 and xrs-6 cells also need to be irradiated for longer periods of time to determine if any changes in cell proliferation eventually occur.

Humans are constantly exposed to low dose rates of radiation. The current LNT model endorsed for regular regulation assumes that no dose of radiation is safe. However, there is a significant amount of evidence that low doses and low dose rates of radiation are beneficial. More studies are necessary to understand the true effects of ionizing radiation at low levels as the results will have crucial radiation regulation

and public health implications.

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