BIOL 3520 RESEARCH PRESENTATION:

Effect of high D-galactose concentrations on the proliferation and viability of Ea.hy926 endothelial cells

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INTRODUCTION

Endothelial cell line

- Endothelial cells contribute the lining of vessels inside the mammalian body.
- Using cancerous cells for research allows for rapid proliferation and reaction.
- Have low mitochondrial oxidation rates with high mitochondrial flexibility for adaption.
- Mitochondrial network is crucial to endothelial function, but choice of energy substrate is vital.

D-galactose

- Monosaccharide sugar, epimer of glucose.
- Often used in media meant for culture in uncontrolled CO_2 conditions.
- Metabolised differently than glucose.
- Known to enhance mitochondrial metabolism, by increased oxygen consumption rate (aerobic respiration).

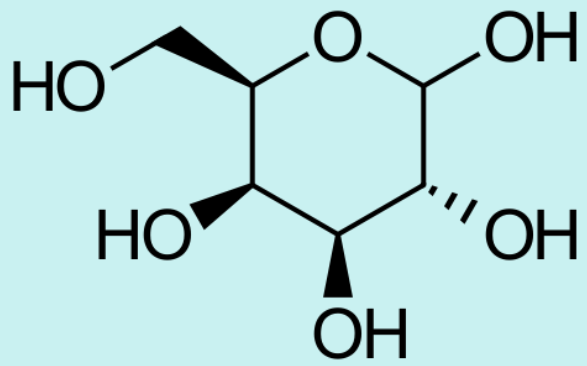


Figure 1. The structure of the cyclic form of galactose, an epimer of glucose.

INTRODUCTION

Previous Research – Galant, et al.

EXPERIMENTAL

- Studied the effects of galactose and glucose on endothelial metabolism in parallel.
- Endothelial cells were cultured in growth medium and seeded into smaller wells and grown to 90% confluence.
- Cells were changed to a glucose- or galactose-containing media separately.

FINDINGS

- Oxygen consumption was increased in the galactose-exposed cells.
- Galactose-exposed mitochondria were more fused – producing larger networks and greater ATP generation
- Using galactose over glucose provides a more suitable target to study mitochondrial-related processes.

ABSTRACT

Objectives

- To investigate the effects of **galactose concentration on endothelial cell proliferation and viability**.
- This study aims to determine whether varying levels of galactose influence cell growth, survival, and metabolic activity, potentially revealing insights into how high-galactose environments impact endothelial cell function.

Methods

- Ea.Hy926 endothelial cell culture
- Count of live cells
- Cell lysis
- BCA protein assay

Findings

- Increased galactose concentration may cause increased protein expression.
- High galactose concentration may cause some cytotoxicity.

EXPERIMENTAL SETUP

Culture Media

- Leibovitz's L-15 media with 10 % FBS and antibiotic/antimycotic solution.
- Aseptically prepared three media with varying amounts of D-galactose:
  - 5 mmol/L (control)
  - 25 mmol/L (x5)
  - 50 mmol/L (x10)

Cell Cultures

- Subcultured, split evenly into 4x T75 flasks, and incubated at 37 °C for 1 week.
- Fed with varying D-galactose concentrations.
- Cultures were incubated for 2 weeks at 37 °C with uncontrolled CO_2 conditions.

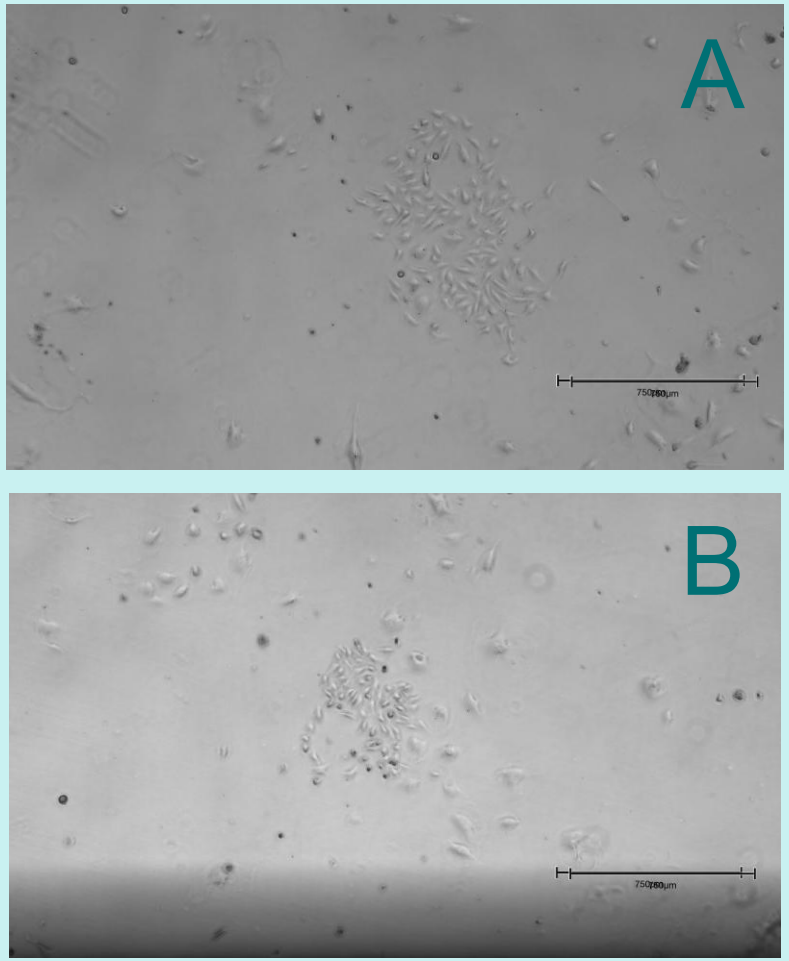


Figure 2. Experimental cell cultures exposed to 25 mmol/L (A) or 50 mmol/L (B) D-galactose visualized using inverted microscope.

METHODOLOGY

Cell Counts:

Counted live cells using trypan blue dye and hemocytometer to measure cell viability when exposed to high concentrations of galactose. Counts were done for 2 replicates.

Bicinchoninic acid (BCA) Assay:

Used to determine cell proliferation based on protein content. RIPA lysis buffer used to release total protein into solution. Samples were prepared from an 8:1 ratio of lysate:BCA working reagent and incubated at 60 ^{o}C for 5 minutes. Standard and sample absorbances were read in triplicate using nanodrop (562 nm).

Scratch Wound Assay:

24 well plate was seeded but did not achieve confluent growth.

Subculture and feed cells with 5, 25, or 50 mmol/L D- galactose

2-week incubation at 37 °C

Cell counting and BCA Protein Assay

Nanodrop measurements and data analysis

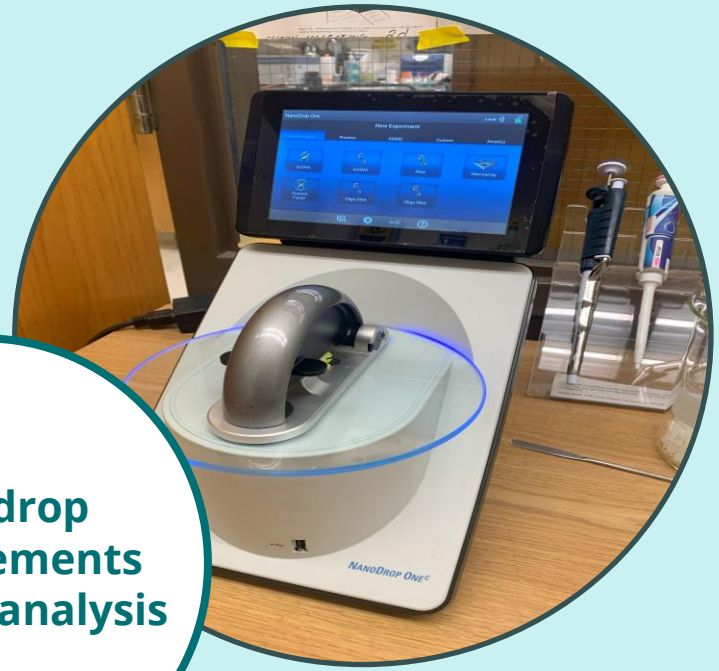
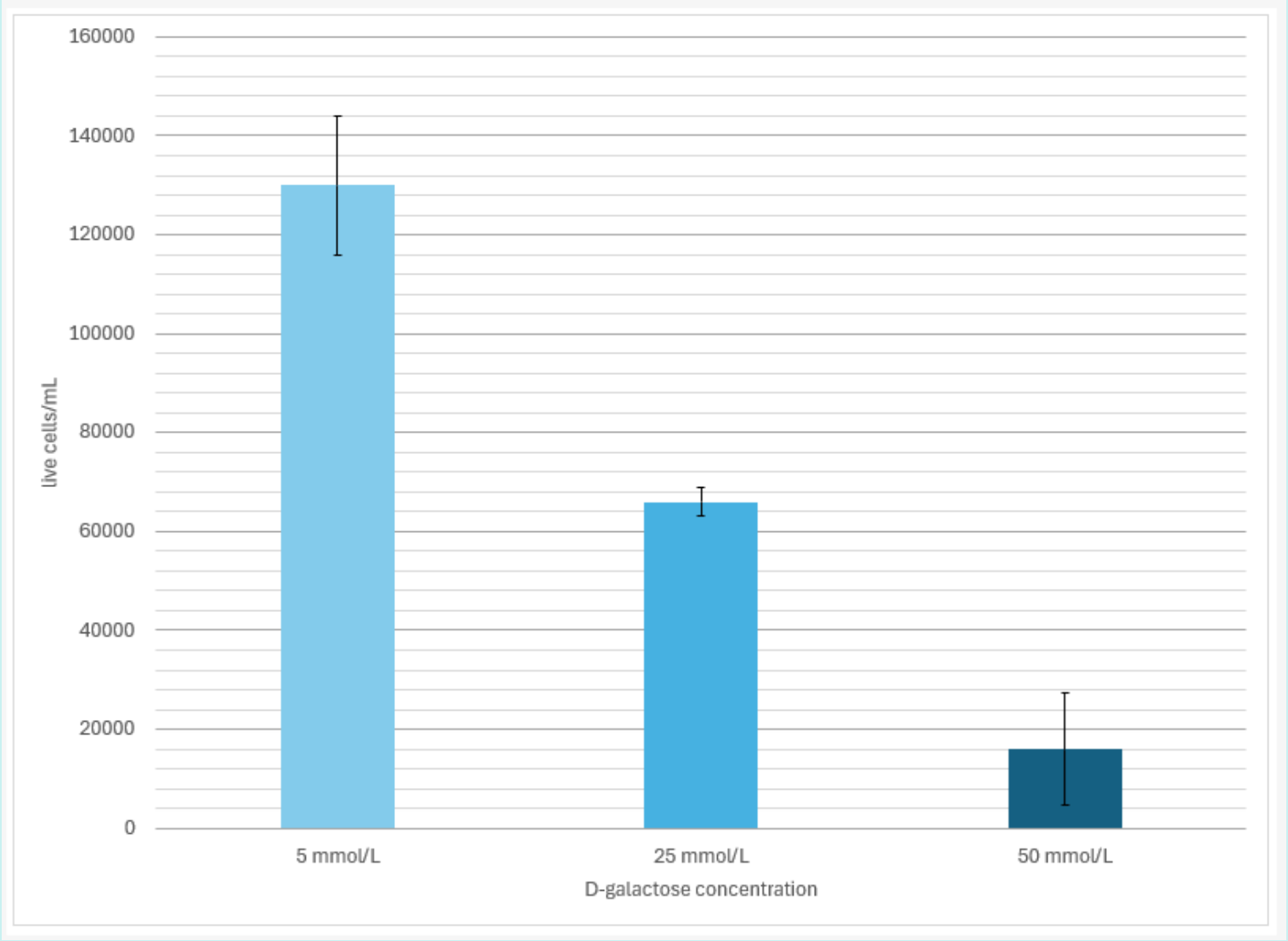


Figure 3: Nanodrop instrument measured absorbance at 562nm

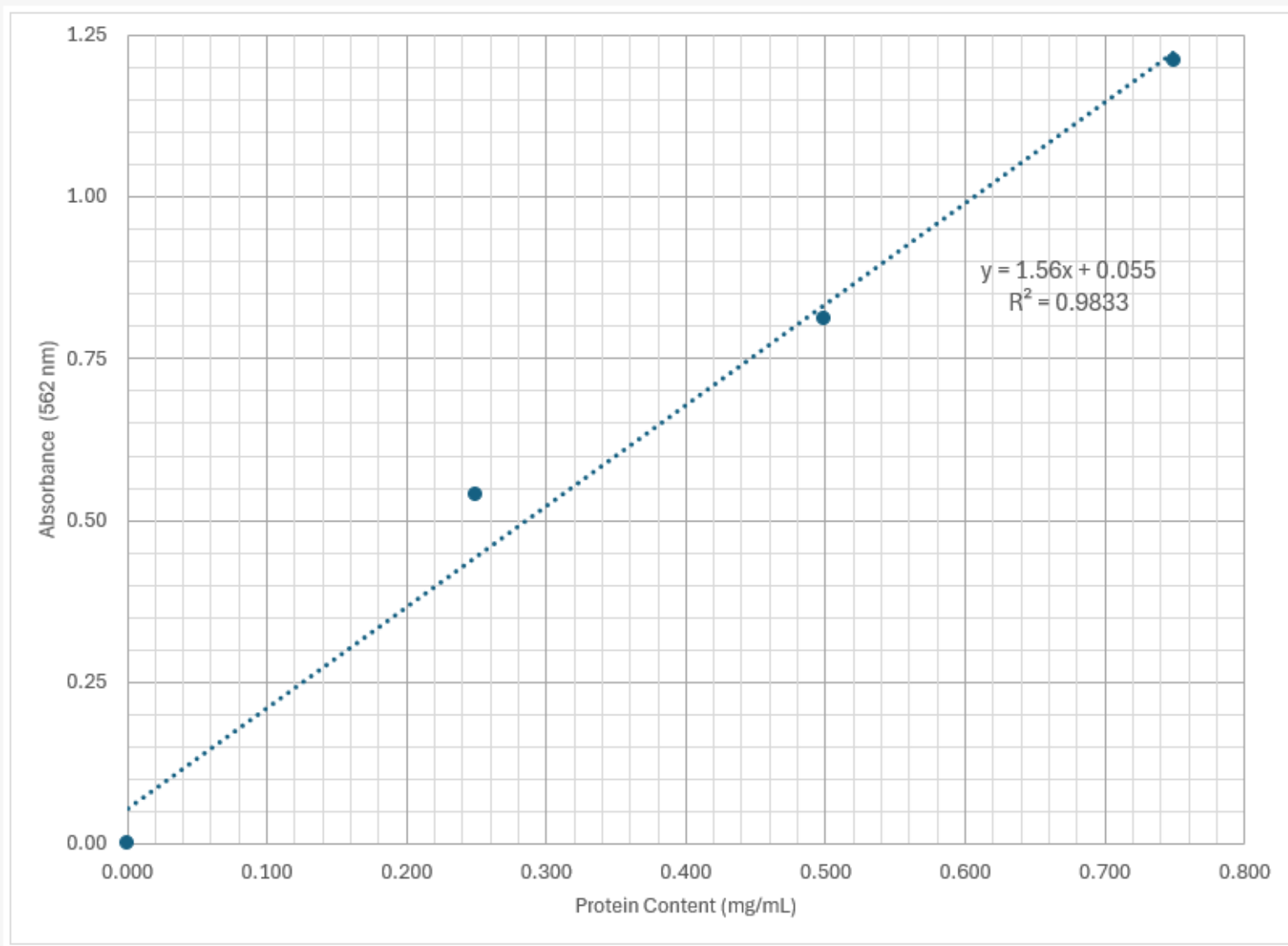
RESULTS – Cell Counts



- Cell viability decreased as concentration of D-galactose increased.
- Data presented as mean ± standard deviation of the mean for two replicate cell counts.

Figure 4. Live cells/mL counted using hemocytometer for Ea.hy926 endothelial cell cultures exposed to various concentrations of D-galactose in culture medium (n = 2).

RESULTS - BCA Assay



- 1.00 mg/mL standard was an outlier and excluded.
- Standard curve used to calculate protein content for experimental cultures
- Samples lied between 0 and 0.1 (edges of linear region)

Figure 5: BCA assay standard curve displaying protein content in mg/mL as average absorbance at 562 nm increases (n=3).

RESULTS – Summary

Table 1. Cell count and BCA assay results showing cell viability and proliferation respectively when Ea.hy926 endothelial cells are exposed to varying concentrations of D-galactose.

Media D-galactose concentration (mmol/L)	Average cells/mL (n = 2)	Average protein content in mg/mL (n = 3)	95 % confidence interval for protein content
5	130,000 ± 14,142.14	0.078 ± 0.007	0.060 - 0.096
25	66,000 ± 2,828.43	0.129 ± 0.003	0.120 - 0.138
50	16,000 ± 11,313.71	$0.022 \pm 4.25 \times 10^{-17}$	0.022 - 0.22

- Overall cell viability decreased as D-galactose concentration increased, but the same trend was not seen for cell proliferation.
- Low standard deviations for protein content indicate high precision.
- Samples were not in the center of linear range of standard curve so results may not be accurate.

DISCUSSION

- Cells with the highest exposure of D-galactose showed very low cell count (16, 000 cells/mL) and protein content.
- Cells exposed to 25 mol/L D-galactose showed low cell count (66, 000 cells/mL) and the highest protein content (0.129 mg/mL)

The moderate galactose culture expressing the highest protein content could be evidence of greater metabolic protein expression in response to galactose, while the high galactose culture showing reduced viability could be evidence of galactose induced cytotoxicity.

Previous Research

- Because galactose promotes oxidative metabolism over anaerobic metabolism, cells grown in galactose-containing media increase their oxygen consumption leading to increased susceptibility to mitochondrial toxins (Galant et al., 2024).
- Generally, focused on the impact of galactose on one aspect function and/or direct comparison to glucose.
- To be supported by literature studies, our findings would need to include other data including specific protein activity etc.

DISCUSSION

Sources of Error

Poor growth conditions

- Evidence of galactose precipitate formation in media.
- Precipitate can cause chelation of other solutes and disrupt cell uptake of all nutrients in media.
- Confluence not attained after 14 days of growth in any of the three cultures.

BCA assay

- Inconsistent preparation of standards
- Inconsistent incubation of standards/samples with BCA reagent
- Sample results lie far from center of standard curve

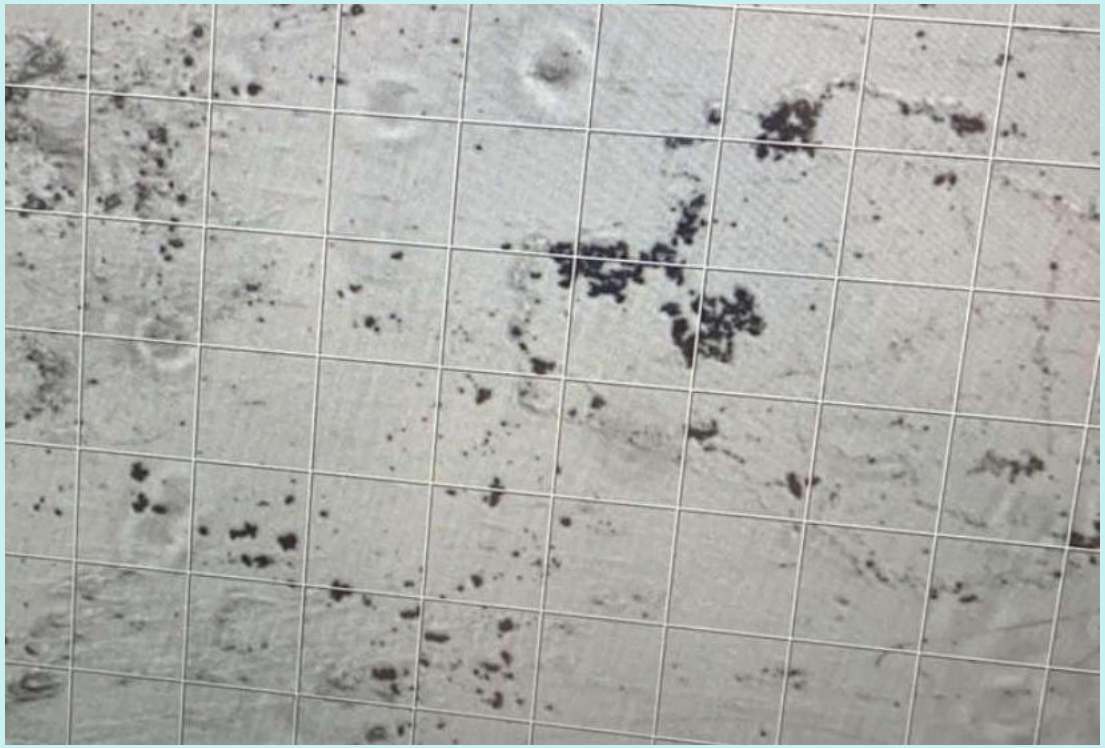


Figure 3. Inverted microscope visualization of the 25 mmol/L galactose culture after 7 days. Galactose precipitate appears as dark spots.

FUTURE WORK

- Produce standard curve with lower concentration protein standards or resuspend cell pellet with < 1 mL PBS so unknowns samples land in the center of the linear region.
- Perform scratch test assay with varying D-galactose concentrations.
- Perform entire experiment again for triplicate days.
- Could perform experiment again with more concentrations of galactose to determine exactly what concentration it becomes detrimental to cell health.

- Larger scope: investigating the metabolic differences of cells cultured in a glucose media vs a galactose media at varying concentrations.

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