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Stem Cell Lab

I. INTRODUCTION

Stem cells are considered one of the primary components of successful regenerative medicine/tissue engineering. These cells have demonstrated the reproductive potential and plasticity required to regenerate viable tissue cell populations. At this point, researchers are racing to identify viable stem cell sources and to develop the means to extract and employ them in therapy. In addition, stem cell lines are being developed to serve as a model for understanding their basic behaviors and therapeutic potentials.

In this experiment, cell cultures of a myoblastic mouse stem cell line (known as C2C12) were used. A variable was then added to the cells to observe what would happen. The variable used in this experiment was insulin [100 iU/mL]. Insulin causes cells in the liver, skeletal muscles, and fat tissue to take up glucose from the blood. Insulin is primarily used for patients suffering from diabetes. Diabetics produce inadequate amounts of insulin and require insulin injections in order to survive. The purpose of this experiment was to determine the effects of an environmental variable, in this case insulin, on the differentiation of these cells.

II. Materials and Methods (Refer to lab sheet)

III. RESULTS

Determining concentration of insulin in high and low plates.

The insulin came in the package as 100 iU/ mL. The stock solution consisted of 0.05 mL of insulin in 4.95 mL of deionized water creating a 1 iU/mL solution of insulin.

$\frac{100\ iU}{1\ mL} \times \frac{0.05\ mL}{5\ mL} = 1\ iU/mL$

High [insulin] plate- 25 µl (0.0250 mL) of stock solution pipetted into 3 mL of media creates a 8.264×10^{-3} iU/mL concentration of insulin.

$\frac{1\ iU}{mL} \times \frac{0.0250\ mL}{3.0250\ mL} = 8.264 \times 10^{-3}\ iU/mL$

Low [insulin] plate- 15 µl (0.0150 mL) of stock pipetted into 3 mL of media creates a 4.975×10^{-3} iU/mL concentration of insulin.

$\frac{1\ iU}{mL} \times \frac{0.0150\ mL}{3.0150\ mL} = 4.975 \times 10^{-3}\ iU/mL$

	Control (10% media)	1% media, no insulin	1% media, low [insulin]	1% media, high [insulin]
Confluency	Very low. Just a few cells. 5% confluency	75% confluency	80% confluency	90% confluency
Differentiation	No differentiation	Little differentiation.	Some differentiation; a couple of myotubes have formed.	Lots of differentiation; many myotubes which were highly developed

IV. CONCLUSIONS

The results of this experiment seem to suggest that insulin had a significant effect on the growth of C2C12 muscle stem cells. Since there was only qualitative data, a statistical analysis would not tell if it was a significant change. To further the experiment, however, a differentiation assay could be used to find quantitative data. Because the differentiated cells have a different structure than the original cells, they can be distinguished from non-differentiated cells. One would somehow have to label the cells in the culture that have differentiated. This could possibly be done by applying a radioactive isotope that codes for something that is found in only differentiated cells, such as myotubes. Once the myotubes have been radioactively labeled, one could count them and get quantitative results. In order to get more accurate results, more trials were needed. The results were from a mouse cell line, so the results may vary for a human trial.