The Effects of Folic Acid on Zebrafish Development

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Abstract

Folic acid is a common prenatal vitamin used to help with neural and cardiac development in babies. In this experiment, zebrafish were used as a model organism due to the genetic similarities between them and humans. The purpose of this lab was to investigate the effects of folic acid of varying concentrations on the embryonic development of zebrafish (*Danio rerio*). It was hypothesized that a daily dose of folic acid would aid the zebrafish embryos in proper development and growth of cardiac and neural elements. The hypothesis was supported by the experiment.

The placement of developing zebrafish embryos in various concentrations of folic acid solution resembles the exposure to folic acid a human embryo would took that amount of folic acid. Zebrafish embryos were placed in wells with a solution of either a daily dose of folic acid or a solution 500x more concentrated. The embryos were observed daily and the stage of their development was catalogued, and solutions were replaced.

Pregnant women are advised to take folic acid daily. Based on the results of this lab, this practice can be supported, due to the improved health of the embryos exposed to folic acid.

Introduction

Folic acid plays a necessary role in neural and spinal development. In the folate mediated one-carbon cycle, folic acid "is a cofactor in one-carbon metabolism, acting as a shuttle for methyl groups that will be used in the metabolism of s-adenosyl methionine (SAM), *de novo* synthesis of purines and thymidylate" (Lee et al., 2012).

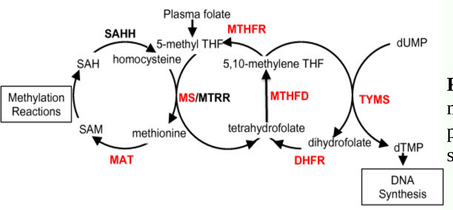

Figure 1: This image shows the complete mediated one-carbon cycle for folic acid in a person. When this cycle is completed, DNA synthesis is initiated (Lee et al., 2012).

As shown in **Figure 1**, DNA synthesis leads to gene expression for neural and cardiac development.

A pregnant woman is advised to take 460 µg of folic acid to prevent miscarriages and spina bifida. The average weight of a woman in 2010 was 75.6 kilograms, compared to an average zebrafish weight of 240 micrograms. The equivalent folic acid dose in zebrafish is $1.46 * 10^{-6}$ µg. Zebrafish are found in the Ganges River in India. Zebrafish have been used as a model organism in experiments since the 1960s. Their bodies are transparent, and they grow at a fast rate, making them ideal for observation. Zebrafish also share 70% of their genes with humans, and 84% of human diseases have been found to have a zebrafish counterpart.

Materials & Methods

First, a mortar and pestle was used to crush one folic acid pill into a very thin powder. Next, 500mL of dechlorinated water was measured and poured into a 500mL beaker, and was repeated for another 500mL beaker. 5mL of dechlorinated water was measured and poured into the 100mL beaker. The crushed folic acid pill was then added to one of the 500mL beakers of dechlorinated water and stirred very well. 1mL of the newly mixed folic acid solution was added to the 100mL beaker and stirred. 2.5mL of dechlorinated water in the final 500mL beaker were removed and finally, 2.5mL of the folic acid solution in the 100mL beaker were added to the 500mL beaker containing 497.5 mL of dechlorinated water and mixed well. The concentration five-hundred times greater was created following a similar procedure, but only one 500 mL beaker was filled with dechlorinated water and the crushed folic acid pill was added to the 100mL beaker. Next, 5 healthy embryos were transferred into each well. 3.5 milliliters of the appropriate solution was added to each well. Solutions were covered, and the tray was then capped and placed into the incubator at 28.5 degrees Celsius over night.

The final part of the experiment was the observations and solution exchanges. The well-tray was removed from the incubator. The embryos were then observed under the stereoscope and tallied under the stage they had reached in hours post fertilization. Any dead embryos were tallied under the "death" column and then removed with a wide bore pipette. Solutions were then exchanged with fresh ones. Once all solutions were exchanged the tray was placed back inside of the incubator. The procedure for this part of the experiment was repeated over the next three days.

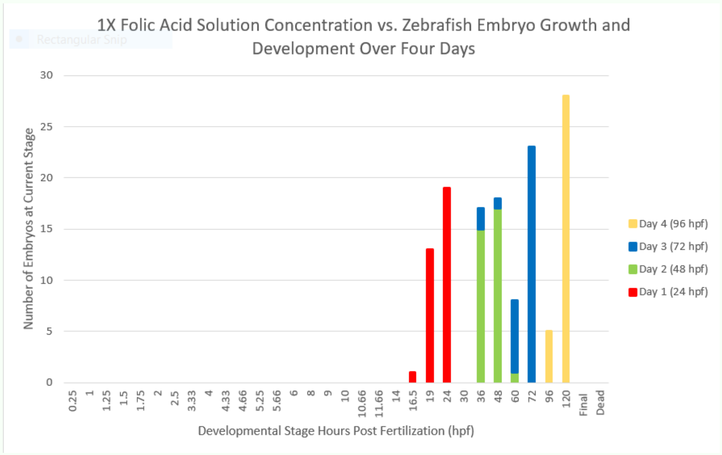

Figure 3: 1X Folic Acid Solution Concentration vs. Zebrafish Embryo Growth and Development Over Four Days. The graph displays the development of the zebrafish embryos in the single dose of Folic Acid. The control is represented by each day. On day one, many of the zebrafish are behind on development. Over days two and three, on average, they appear to reach the control group. On the final day, a majority are ahead of the control group.

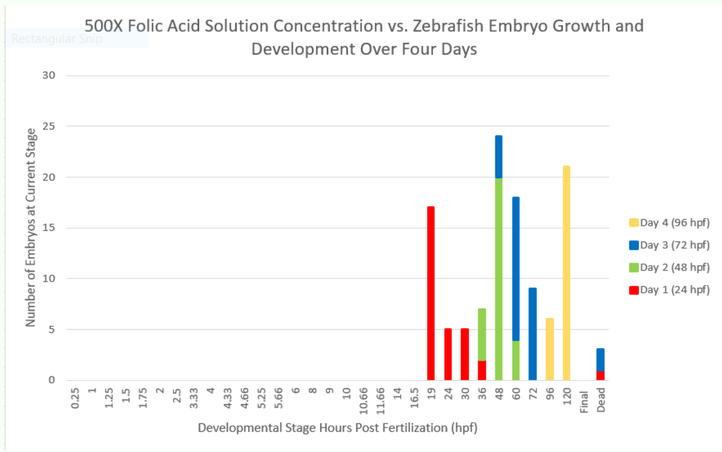

Figure 4: 500X Folic Acid Solution vs. Zebrafish Embryo Growth and Development Over Four Days. The graph above shows the development of the zebrafish embryos in the 500X folic acid dose. On day one, about half of the embryos acid solution. On day two, the average amount of embryos appear to be at the control group level. On day three, they appear to be slightly below or at the control level. Similar to the 1X Folic Acid Solution, a majority of the zebrafish appear to be above the control group on the fourth day.

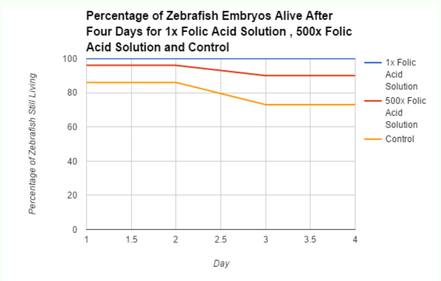

Table 5: The percentage of embryos that remained living at the end of the lab are displayed for 2x Folic Acid Solution, 500x Folic acid solution, and the control group.

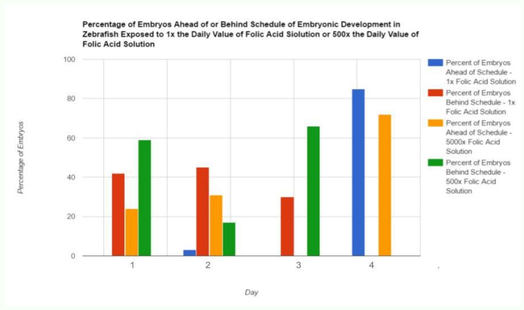

Figure 6: A graphical representation of how many fish were ahead of or behind the normal development schedule

Results

The experiment was performed by introducing the embryos to either a single dose concentration of folic acid or five hundred times a single dose concentration. The experiment was carried out for four and the data collected reflects the stage each embryo was at during the time of observations, in hours past fertilization (hpf).

The control group for this lab was not exposed to any folic acid and was allowed to develop with no chemical interference. The experimental groups were exposed to varying amounts of folic acid. **Figure 2** provided a comparison of the control and experimental group by acting as a control. The independent variable in this experiment was the amount of folic acid the zebrafish embryos were exposed to. The dependent variable was the zebrafish spinal development and stage (hpf) progression in response to the solution environment.

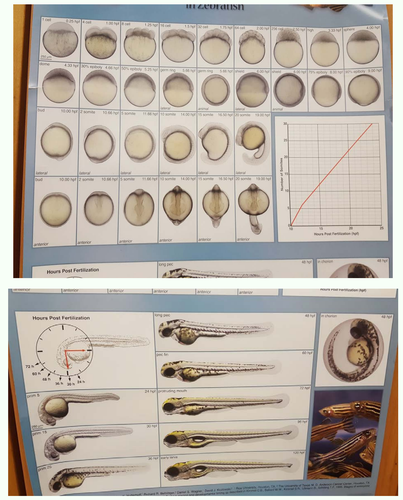

Figure 2: The poster above shows normal zebrafish embryo development. The embryo was stated to remain unhatched until the time between 36 hpf and 48 hpf.

Discussion

The data collected and the results of the experiment support the hypothesis. Both solutions containing folic acid appeared to aid the embryos in development. The three embryo deaths occurred in the 500X Folic Acid Solution and no embryo deaths occurred in the 1X Folic Acid Solution. The 0% mortality rate of the 1X Folic Acid Solution was proven to be statistically significant. All hatched embryos developed proper spines and body parts. The data also shows that folic acid maintained an average rate of progression, and appears to have accelerated the progression of the hatched zebrafish above the normal rate of development for the control group.

Although the experiment yielded satisfactory results, a few minor errors could be corrected to improve the procedure, such as adding a control group and taking observations on Day 0. The limitations of this lab were time and number of embryos available, and more time could have allowed for more observations post hatching. The number of embryos available also was a large limitation as not every lab group could perform a control group, leading to changes among data since amounts of solutions varied among each group.

References

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