The Effect of Food Quality in Growth Medium on Ciliary Regeneration in *Tetrahymena*

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Introduction

Tetrahymena is a type of ciliated, single-celled protozoan that can be found in freshwater environments. These eukaryotes have a complexity similar to that of human cells, proving to be advantageous as a model system for studying macromolecular biogenesis, particularly cilia regeneration (4). Cilia are short, hairlike structures that surround the membrane of the *Tetrahymena* cell in multiple rows (1). Containing roughly 800 cilia per cell, the *Tetrahymena* uses its cilia for movement and eating (5). Because the cilia consist of proteins and microtubules, *Tetrahymena* require protein synthesis and the assembly of microtubule proteins such as tubulin in order to perform ciliary regeneration (3). When starved, *Tetrahymena* cells synthesize protein at a slower rate than normal, growing cells. However, after the deciliation of starved cells, *Tetrahymena* have been known to increase their rate of protein synthesis, often producing slightly more protein than required to maintain a starved cell and regenerate cilia (2).

The goal for this lab was to observe how the quality of food in the growth medium affects the amount of time it takes *Tetrahymena* regenerate its cilia after complete deciliation. It was hypothesized that the *Tetrahymena* population in the growth medium with the largest food concentration (undiluted, normal NEFF medium) will have the fastest ciliary regeneration.

Materials and Methods

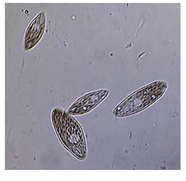

Figure 1. Photograph of *Tetrahymena* at 10x magnification (Taken using MotiCam)

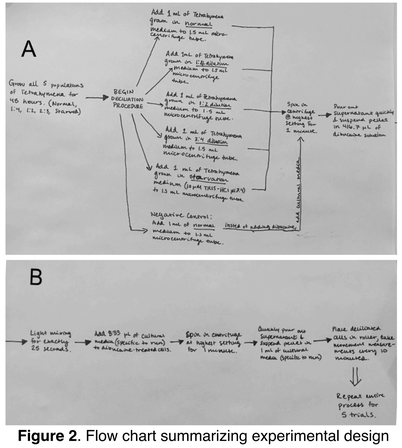

Figure 2. Flow chart summarizing experimental design and highlighting deciliation process. (A) First part of flow chart. (B) Continuation of flow chart.

Results

For all types of growth media, the *Tetrahymena* cells recovered motility and regenerated cilia in about the same amount of time and roughly at the same rate, although there were some disparities between certain populations at varying times. When comparing the *Tetrahymena* grown in normal media to the starved *Tetrahymena* during minutes 60-80, there is a significant difference in the rate of ciliary regeneration ($p<0.05$). Even though there is a difference in rate present during this time interval, both populations of *Tetrahymena* still attain full ciliary regeneration within the same amount of time (90 minutes) in the end. Thus, there was no overall significant difference across all populations of *Tetrahymena* when comparing % motility with respect to time (Table 1). The results from Figure 4, however, reveal the relationship between time and % motility that is fairly consistent across all growth media types. The trendlines for all growth media types appear to follow a slight exponential curve and then levels off once the cell population nears 100% motility. There is slow ciliary regeneration for the first 40 minutes after deciliation, and then there is an increasing rate of ciliary regeneration between 40-65 minutes. At the 65 minute mark, the graph appears to level off as the rate begins to slow down until almost full motility is gained after 90 minutes (Figure 4).

There was also an observable difference between ciliated, deciliated, and dead cells that was discovered during this experiment (Figure 3). Fully ciliated cells move in a spiral swimming pattern and maintain a pyriform shape (1). Fully deciliated cells either barely move in place or are completely still and have a slightly more rounded and oval shape than ciliated cells. Dead cells do not move at all and will either fail to regenerate new cilia or will undergo lysis, which is the breaking down of the cell membrane (4).

Table 1. T-tests comparing all the varying populations of *Tetrahymena* to each other. All *p*-values > 0.05; no populations are significantly different from each other.

Populations Being Compared	*p*-value
Normal vs. Starved	0.5110
Normal vs. 1:4 Dilution	0.6455
Normal vs. 1:2 Dilution	0.7138
Normal vs. 3:4 Dilution	0.9784
Starved vs. 1:4 Dilution	0.8225
Starved vs. 1:2 Dilution	0.7659
Starved vs. 3:4 Dilution	0.5134
1:4 Dilution vs. 1:2 Dilution	0.9330
1:2 Dilution vs. 3:4 Dilution	0.7251
1:4 Dilution vs. 3:4 Dilution	0.6538

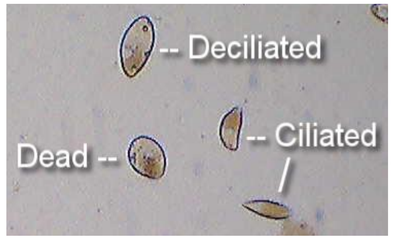

Figure 3. Labeled photograph of ciliated, deciliated, and dead *Tetrahymena* at 10x magnification (Taken using MotiCam)

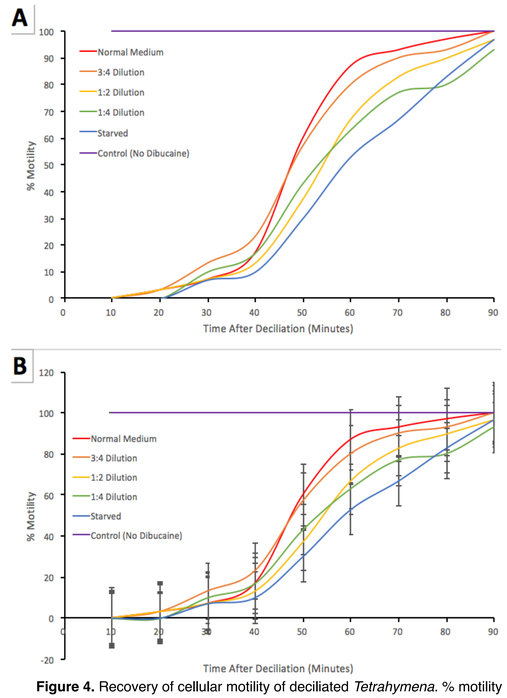

Figure 4. Recovery of cellular motility of deciliated *Tetrahymena*. % motility measured by the mean (n=5) percentage of cells (±AAD) that have recovered full motility. Treatments: Normal medium, 3:4 dilution medium, 1:2 dilution, 1:4 dilution, starved, and negative control (no dibucaine added). Dibucaine concentration was controlled with 2 mg of dibucaine per 4 mL of medium. % motility is the % of cells in a sample that have regained full swimming behavior. (A) No uncertainty bars present and smaller axes range for more in-depth graph. (B) Greater axes range to include uncertainty bars.

Conclusions

The experiment's outcome does not support the hypothesis that the Tetrahymena population in the growth medium with the largest food concentration (undiluted, normal NEFF medium) will have the fastest ciliary regeneration. The actual outcome exhibited that Tetrahymena cells recovered motility and regenerated cilia in about the same amount of time and roughly at the same rate in the end, showing no significant difference overall since all p-values > 0.05 (Table 1). The results of this lab occurred rather than hypothesized results possibly because after the deciliation of starved cells, Tetrahymena tend to increase their rate of protein synthesis, producing slightly more protein than required to maintain a starved cell and regenerate cilia; the significance of this extra protein synthesis is currently unknown (2). This fact disproves the hypothesis and its rationale that a growth medium with larger food concentration/quality will have the fastest cilia regeneration because with a greater food source, there is more energy for the cell to use and that because there is more energy, more proteins will be synthesized for cilia regeneration.

Major sources of uncertainty in this lab resulted from the amount of dibucaine in the dibucaine solution, determining the normal speed of the Tetrahymena after deciliation, and counting the cells after deciliation. The amount of dibucaine required to make the solution was 2 mg, which is a very small amount when attempting to mass milligrams on a gram scale. To eliminate this source of uncertainty, a larger mass of dibucaine, ideally one gram or greater, could have been used instead. In addition, deciding when a Tetrahymena cell has reached its normal speed after deciliation often proved difficult because it was required that each cell in motion was timed from start and end markings on the capillary tube in order to calculate its speed, which lacks efficiency and precision since there are a multitude of cells. In order to prevent an inaccurate result, the deciliated Tetrahymena could be diluted with distilled water so that less cells are being observed, making it easier to measure their speed. This change in protocol for measuring speed could be useful in reducing uncertainty when counting cells after deciliation as well.

As presented by the results, the outcome of this experiment may not support the hypothesis, but significant trends were discovered: Tetrahymena cells recovered motility and regenerated cilia in about the same amount of time and same rate in the end, and that the cells' ability to overcome total loss of cilia even when starved exhibits that this system proves to be advantageous as a model for studying macromolecular biogenesis.

Literature Cited

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For Further Information

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