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

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Edgar, M., Duckweed Tigers, 04/27/18

Abstract: The goal of this experiment 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, quantified as % motility vs. time. The food quality was varied by making different dilutions of the normal growth (NEFF) medium or by using a starvation growth medium in order to observe ciliary regeneration under various food conditions. Movement measurements were taken every ten minutes, and the number of cells that had reached normal speed and swimming behavior was indicated. Calculations were then performed in order to find % motility at each ten minute interval. Based on the collected data, *Tetrahymena* cells of all growth media types recovered motility and regenerated cilia in about the same amount of time (90 minutes) and same rate (between 1.32 and 1.54% motility/min) overall. In addition, observed trend lines show that there was slow ciliary regeneration for the first 40 minutes after deciliation, and then there was 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 was gained after 90 minutes. Because starved *Tetrahymena* tend to increase their rate of protein synthesis after deciliation, they produce slightly more protein than required to maintain a starved cell and regenerate cilia, thus serving as rationale as to why *Tetrahymena* can recover motility in the same amount of time and at the same rate despite receiving a lesser food quality as seen in the lab results.

Introduction: *Tetrahymena* is a type of ciliated, single-celled protozoan that can be found in freshwater streams, lakes, and ponds. 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.[1] Cilia are short, hairlike structures that surround the membrane of the *Tetrahymena* cell in multiple rows.[2] Containing roughly 800 cilia per cell, the *Tetrahymena* uses its cilia for movement and eating.[3] The alternating vibrations and bending of the cilia create a spiral swimming pattern, causing currents in the surrounding fluid in order to propel the cell forward. The cilia also use these currents to move food particles into the cytostome, or mouth, of the cell[4]. By combining in very specific arrangements, microtubules make up the cilia surrounding the cell. Microtubules are strong and thick spirals of thousands of subunits, and these subunits are made up of a protein known as tubulin.[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.[6] Cilia tend to fully grow back within 70-90 minutes after amputation; full ciliary regeneration is defined as when the cells recover motility and return to their normal swimming behavior/speed.[7] When starved, *Tetrahymena* cells synthesize protein at a slower rate than normal, growing cells. However, after the deciliation of starved cells, *Tetrahymena* often increase their rate of protein synthesis, often producing slightly more protein than required to maintain a starved cell and regenerate cilia.[8]

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[1] Skriver, L., and Williams, N. E. 1980. Regeneration of cilia in starved *Tetrahymena thermophila* involves induced synthesis of ciliary proteins but not synthesis of membrane lipids. Biochem. J 188:695-704.
[2]Bayless, B. A., et al. 2016. *Tetrahymena* basal bodies. BioMed Central 5:1-5.
[3] Skriver, L., 188:695-704.
[4] Bayless, B. A., 5:1-5.
[5] Rosenbaum, J. L., and Carlson, K. 1969. Cilia regeneration in *Tetrahymena* and its inhibition by colchicine. J. Cell Biol 40:415--425.
[6] Rannestad, J. 1974. The regeneration of cilia in partially deciliated Tetrahymena. J. Cell Biol 63: 1009-1017.
[7] Rosenbaum, J. L., 40:415--425.
[8] Calzone, F. J., Angerer, R. C., and Gorovsky, M. A. 1983. Regulation of protein synthesis in *Tetrahymena*: quantitative estimates of the parameters determining the rates of protein synthesis in growing, starved, and starved-deciliated cells. The Journal of Biological Chemistry 258(11):6887-6898.

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, quantified as % motility vs. time. 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: In order to evaluate the hypothesis that the *Tetrahymena* population in the growth medium with the largest food concentration will have the fastest ciliary regeneration, the food quality was varied by making different dilutions of the original normal growth (NEFF) medium, using the normal growth medium, and by starving the *Tetrahymena* from normal growth medium for 24 hours prior to deciliation in 10μM TRIS-HCl pH 7.4. Because there was only 1 M TRIS-HCl pH 7.4 available, the starvation media was diluted to decrease concentration down to 10μM, which was determined to be the ideal concentration to starve cells in preliminary. The concentration of starvation media was controlled so that some cell populations are not negatively effected or more starved than others. The varying dilutions of the original NEFF medium were a 1:4 dilution (6.25 ml original NEFF, 18.75 ml dH_2O), 1:2 dilution (12.5 ml original NEFF, 12.5 ml dH_2O), and 3:4 dilution (18.75 ml original NEFF, 6.25 ml dH_2O). All diluted NEFF media were made in preliminary testing and were also used in final testing. Through preliminary testing, the best method for carrying out this experiment was determined. The populations of *Tetrahymena* were quantified using a hemocytometer (average 57777.8 cells/mL), the normal, average speed of the cells was measured using a capillary tube (average 0.466 mm/sec), and the most ideal starvation and deciliation procedures were determined. There were many difficulties in determining the deciliation procedure because it is very particular and time-based. It was discovered through preliminary testing that mixing times with dibucaine and concentrations of dibucaine had to be decreased, thus allowing for these changes to be made for final testing in order to deciliate the cells. The lab was set up by first growing the *Tetrahymena* in their specific medium for 48 hours. The amount of time allowed for cell growth in medium was controlled so that roughly the same number of cells were being produced for each population as a means to isolate the effects of food quality of growth medium on ciliary regeneration. There were five trials in total, with the five treatment groups/runs being *Tetrahymena* cells grown in the normal NEFF media, cells grown in 3:4 dilution of NEFF media, cells grown in 1:2 dilution of NEFF media, cells grown in 1:4 dilution of NEFF media, and cells grown in starvation media of 10μM TRIS-HCl pH 7.4.

After allowing the *Tetrahymena* populations to grow for two days, the deciliation procedure was carried out. THe entirety of the deciliation process was controlled by using the same volume amount and time amount required at each step as a means to produce viable, deciliated cells for observation. First, 1 mL of the *Tetrahymena* culture was added to a 1.5 mL microcentrifuge tube with a curved bottom and spun in a centrifuge at the highest setting for one minute. The curved bottom tube was used because after deciliation, the cells tend to be very fragile so using a curved bottom makes it easier to resuspend the cells after centrifugation without damaging them. The supernatant was then quickly poured off and the pellet was suspended in 416.7 μL of the dibucaine solution with light mixing for exactly 25 seconds. The dibucaine solution was made by adding 2 mg of dibucaine powder to 4 mL of cultural media specific to the run being performed; the dibucaine solution was made fresh for every run and trial. Dibucaine was used to deciliate the cells based on the recommendation of multiple articles (see all cited literature). The negative control was produced by adding cultural media instead of dibucaine solution to the pellet of cells for each run. This control was used to ensure that the dibucaine solution was causing the deciliation of cells and that the centrifugation was not having an effect on the cells that would alter the results. The negative control was expected to have zero deciliated cells. The dibucaine solution was controlled so that all cells were receiving the same concentration of dibucaine in order to ensure that the differences in time taken for ciliary regeneration were not caused by some cell populations only being partially deciliated rather than completely. The amount of time that the cells are in contact with only the dibucaine solution (25 seconds) was controlled as a means to deciliate the cells in the same manner and amount each time. If the concentration of the dibucaine solution was too strong or the amount of time allowed for light mixing with the dibucaine solution was too long, then the cells would not survive

and would undergo lysis. After 25 seconds of light mixing, 833 µL of cultural media was added to the dibucaine treated cells and centrifuged at the highest setting for one minute. The supernatant was quickly poured off and the pellet was suspended in 1 mL of cultural media. The deciliated cells were then placed in a roller to allow for light mixing, with movement measurements taken every ten minutes.

To observe the cells under a compound microscope, they were placed in a capillary tube fixed onto a microscope slide as a means to measure the speed of the cells once they begin moving to determine when the cells have returned to their normal speed and swimming behavior (Figure 1). A cell was determined to have returned to a normal speed when its speed was very similar to or exactly 0.466 mm/sec, in which this value was determined in preliminary testing. Speed was measured by timing how long it took the cell to travel a 3 mm distance, in which multiple 3 mm intervals were marked off on the capillary tube, and then using the formula speed=distance/time (Figure 1). 30 random cells were counted at each movement measurement, and the number of cells that had reached normal speed and swimming behavior was indicated. Calculations were then performed in order to find % motility at each ten minute interval, meaning the number of cells that had returned to their normal speed and swimming behavior. Since the data was quantitative, multiple t-tests were chosen to evaluate significance, comparing all varying growth media curves to each other as well as an individual interval of the growth media curves to give a more in depth insight.

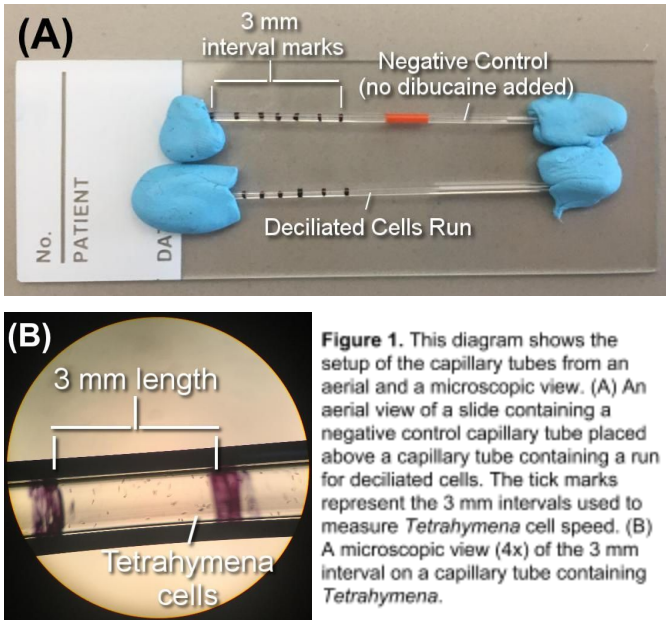


Figure 1. This diagram shows the setup of the capillary tubes from an aerial and a microscopic view. (A) An aerial view of a slide containing a negative control capillary tube placed above a capillary tube containing a run for deciliated cells. The tick marks represent the 3 mm intervals used to measure *Tetrahymena* cell speed. (B) A microscopic view (4x) of the 3 mm interval on a capillary tube containing *Tetrahymena*.

Results: For all types of growth media, the *Tetrahymena* cells recovered motility and regenerated cilia in about the same amount of time (90 minutes) and roughly at the same rate (between 1.32 and 1.54% motility/min), 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 ($p<0.05$). At 60 minutes, *Tetrahymena* grown in normal media had regained 87% motility, whereas starved *Tetrahymena* had only recovered 53% motility at this point in time. This trend is continued for minute marks of 70 and 80, where normal media *Tetrahymena* has 93% motility at 70 minutes and 97% motility at 80 minutes while starved *Tetrahymena* has 67% motility at 70 minutes and 83% motility at 80 minutes (Figure 2). Even though there is a difference in rate present during this time interval, both populations of *Tetrahymena* still attain near complete ciliary regeneration within the same amount of time (90 minutes) in the end. Thus,

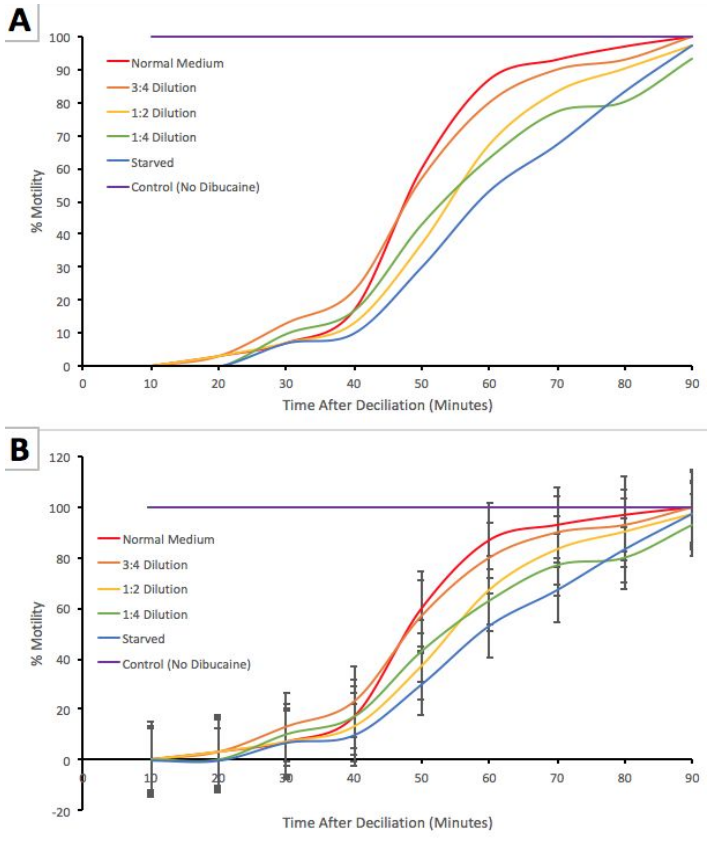


Figure 2. Recovery of cellular motility of deciliated *Tetrahymena*. % motility measured by the mean (n=5) percentage of cells (±AAD) that have recovered full motility. Treatments: Normal medium, 3:4 dilution medium, 1:2 dilution, 1:4 dilution, starved, and negative control (no dibucaine added). (A)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

there was no overall significant difference across all populations of *Tetrahymena* when comparing % motility with respect to time (all *p*-values>0.05).

Tetrahymena, for all types of growth media, recovered motility at roughly the same rate throughout the entire 90 minutes. However, there were some small differences between each of the rates for differing growth media, such as the normal medium producing cells with the greatest rate of motility recovery (1.54% motility gained / min) while the 1:4 dilution medium produces cells with the smallest rate of motility recovery (1.33% motility gained/min) (Table 1). It appears that the greater the concentration of food in the growth medium with the exception of the 1:4 dilution (1.32 rate vs. starvation media's 1.33 rate), the faster the rate of motility recovery will be and thus the faster the rate of cilia regeneration will be (Table 1). Despite this observable trend, the differences between the rates for each growth medium are small enough that they have little to no effect on the overall amount of time it takes for full ciliary regeneration of *Tetrahymena.*

Table 1. Average rate of motility recovery (% motility/min) for each type of growth media. Rate determined using linear trendline function on Excel.

Growth Media	Average Rate of Motility Recovery (% motility/min)
Normal	1.54
3:4 Dilution	1.47
1:2 Dilution	1.43
1:4 Dilution	1.32
Starved	1.33

Additionally, the results from Figure 2 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. For all trendlines present in the graph, there was slow ciliary regeneration for the first 40 minutes after deciliation, and then there was 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 was gained after 90 minutes (Figure 2).

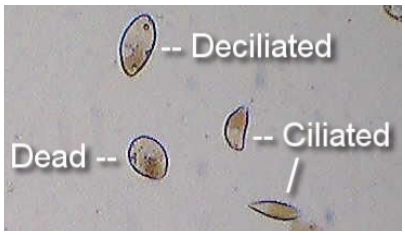


Figure 3. Labeled photograph of ciliated, deciliated, and dead *Tetrahymena* cells at 10x magnification (Taken using MotiCam).

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.[9] 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, after complete detachment of cilia, 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.[10]

Discussion: 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 (90 minutes) and roughly at the same rate (between 1.32 and 1.54% motility/min) in the end, showing no significant difference overall since all *p*-values > 0.05 when comparing the cells from differing growth media (Figure 2, 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.[11] 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

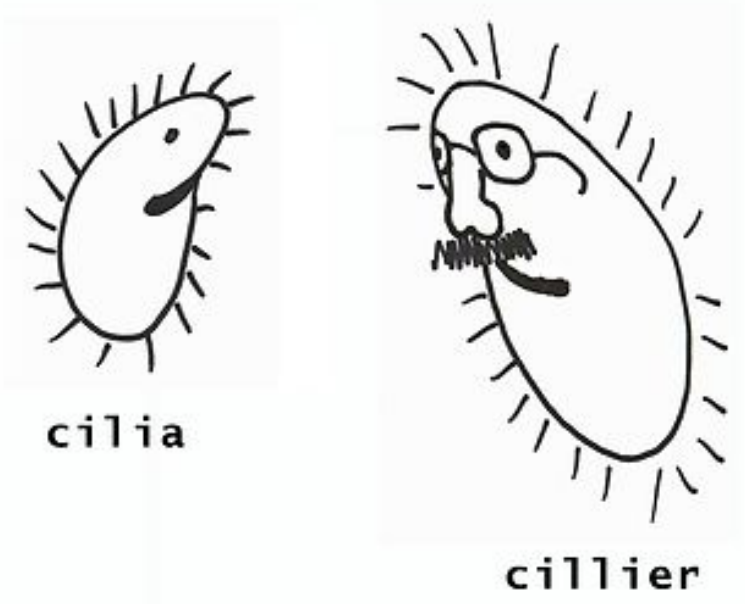
[9] Bayless, B. A., 5:1-5.
[10] Rosenbaum, J. L., 40:415--425.
[11] Calzone, F. J., 258(11):6887-6898.

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 in order to allow for a more precise and accurate measurement.

A further experiment could explore the relationship between protein synthesis and ciliary regeneration in deciliated cells since it was determined from this experiment that protein synthesis plays a major role in biogenesis of ciliary membranes. This future lab could be carried out by varying the type of chemical compound that a *Tetrahymena* population is treated with as means to study each compounds' effects on protein synthesis, cilia regeneration, and resorption of cilia. The chemical compounds to be used are cycloheximide, colchicine, and vinblastine sulfate, as recommended by two different articles.[12][13] However, more trials might need to be performed to perfect the deciliation process through preliminary testing before carrying out future experimentation. In order to make this future experiment viable, a method for decreasing uncertainty to produce more accurate and consistent results would need to be designed. This next step could allow for further exploration into the processes behind ciliogenesis as a means to better understand how these different systems work together.

As presented by the results, the outcome of this experiment may not support the hypothesis, but significant trends were discovered: *Tetrahymena* cells of all growth media types recovered motility and regenerated cilia in about the same amount of time (90 minutes) and same rate (between 1.32 and 1.54% motility/min) overall.



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[12] Rannestad, J., 63: 1009-1017.
[13] Rosenbaum, J. L., 40:415--425.