The effect of temperature on food vacuole formation by *Tetrahymena thermophila*

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Abstract

Tetrahymena thermophila are ciliated protozoa that ingest food through the process of phagocytosis. In an experiment conducted to further study the metabolism of this organism, the effect of temperature on its metabolic rate was examined through the appearance of food vacuoles. Test tubes containing *T. thermophila* cells were exposed to three different treatment temperatures (12° C, 22° C, and 30° C) for 20 minutes each, and the mean number of food vacuoles formed in 10 cells after every 5 minutes within that time interval was subsequently recorded. Our data suggest that an increase in temperature does increase the metabolic rate of *T. thermophila*, as the average number of food vacuoles per cell following exposure to 12° C, 22° C, and 30° C, was 0.4, 1.2, and 2.8 respectively. Since the optimal temperature of this organism ranges from around 28° C to 30° C, we can infer that because of this, the cells appear to metabolize quickest at these temperatures.

Introduction

Phagocytosis is a fundamental metabolic process performed by a number of eukaryotes through which large molecules, e.g. microbes and food particles, are ingested for various purposes such as the maintenance of homeostasis, the activation of the cell's immune system, as the uptake of food to meet the organism's nutritional needs (Jacobs *et al.* 2006, Sugita *et al.* 2009). For *Tetrahymena thermophila*, a unicellular and free-living ciliate, phagocytosis does not only serve as a primary means to obtain nutrients from food, but it is also essential for the disposal of wastes and undigested material from the cell, which is achieved through the formation of food vacuoles (Sugita *et al.* 2009). Microorganisms are consumed by *T. thermophila* cells as a source of food via the oral apparatus, a funnel-like structure located in the anterior portion of the cell that is interiorly lined with cilia to propel the food particles throughout the cell membrane and into the cytoplasm (Gonda *et al.* 2000). As food travels along the oral apparatus, it eventually reaches the innermost portion of the structure called the

cytosome, which functions similar to that of a mouth (Gonda *et al.* 2000). As soon as food reaches the cytosome, it is packaged into smaller membrane-bound organelles known as vacuoles which are subsequently transported into the cytoplasm with the help of deep fibers embedded within the cell membrane (Gonda *et al.* 2000). Once a food vacuole is formed, its nutrients are immediately taken up by the cell. Lysosomes scattered within the cytoplasm fuse with the food vacuole shortly after and release special enzymes that will fully digest the food particle (see Figure 1) (Jacobs *et al*. 2006, Rasmussen 1976). To complete the digestion process, this food vacuole that now consists of wastes and undigested material will then be signalled to leave the cell (Rasmussen 1976).

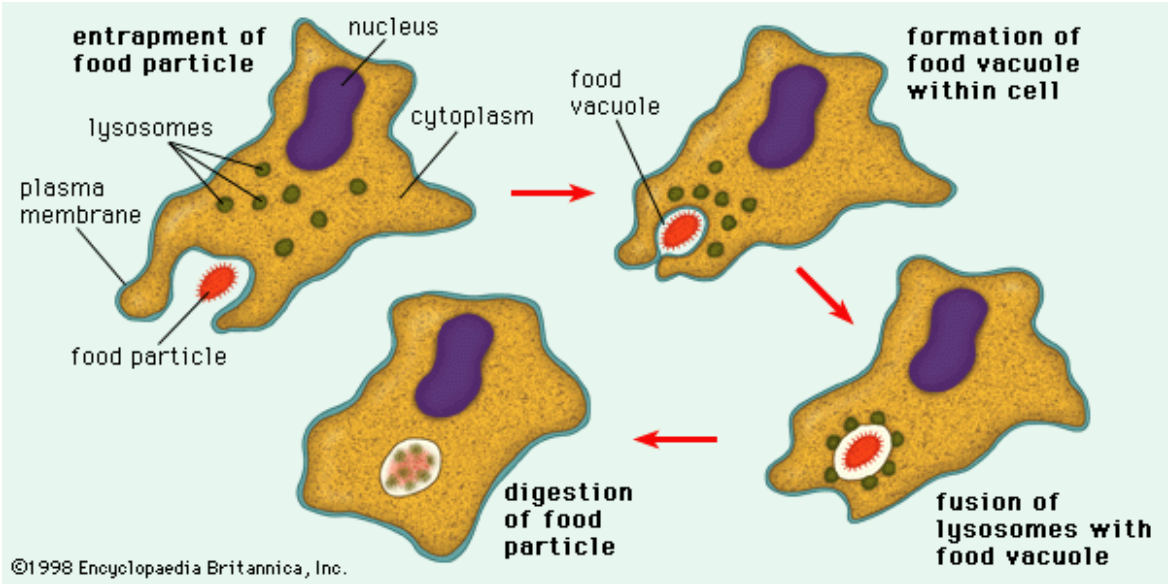

Figure 1. Illustrated is the overall process of phagocytosis throughout an ordinary cell, beginning with the ingestion of the food particle to its digestion by lysosomes. Adapted from Encyclopaedia Britannica, 1998.

Since the process of phagocytosis has proven to be of great importance to the growth and survival of *T. thermophila*, we were interested in studying factors that might affect the metabolism of the organism as measured by the number of food vacuoles formed. Since an increase in temperature enhances the fluidity of the membrane which can consequently help or hinder the function of transport proteins embedded within it (Martin *et al.* 1976), we decided to

focus on temperature as our abiotic factor as an increase or decrease in temperature could potentially affect the process of phagocytosis. In addition, Nagel and Wunderlich (1977) carried out an experiment to study the effect of temperature on RNA-transport throughout cells of a *Tetrahymena* species, and the results had shown that the process occurred much slower at temperatures of 18° C and 23° C than at the organism's optimal temperature of 28° C. With the background information provided on the significant characteristics of the cell membrane, as well as data and observations from experiments like that of Nagel and Wunderlich (1977), we predicted that increasing temperature would enhance the metabolic rate of this organism, since it is apparent that they function best at temperatures that are fairly close to its relatively high optimal temperature. Therefore, our alternate hypothesis states that an increase in temperature will increase the number of food vacuoles formed by *T. thermophila*, while our null hypothesis states that an increase in temperature will decrease or have no effect on the number of food vacuoles formed by *T. thermophila*.

Methods

After swirling the 500mL flask to ensure a proper distribution of *T. thermophila* cells, sterile technique was conducted before we pipetted 4mL of *T. thermophila* cells into a 6mL test tube. We then placed the test tube with 4mL of *T. thermophila* into a 12ºC incubator for 10 minutes. We added 10µL of standard prefer into five 500µL microcentrifuge (mcf) tubes and labelled each tube with the time at five minute intervals from 0-20 minutes as well as the treatment temperature. Air bubbles were removed from the mcf tubes using finger vortex. After 10 minutes in the incubator, we took the test tube out of the incubator and added 1mL of NEFF growth medium with Alizarin Crimson 0102004 (a non-toxic dye) to the test tube. We re-suspended the sample and swirled the solution until the colour was consistent.

We pipetted 150µL of *T. thermophila* out of the test tube into the 500µL mcf tube with 10µL of prefer. This sample is defined as the t=0 time interval. We placed the *T. thermophila* back into the incubator for 5 minutes. We continued to pipette 150µL out of the sample at 12ºC into the corresponding 500µL mcf tube for each five minute time interval until 20 minutes. These procedural steps were repeated three times at 12ºC, 22ºC (control temperature), and 30ºC to make a total of nine replicates. We re-suspended the sample before pipetting 45µL of *T. thermophila* at 0 minutes and 12ºC onto a slide. We placed the slide under a compound microscope and counted the number of cells in the field of view at 100X magnification.

We recorded observations such as the shape of the cells and the size of the food vacuoles. We then counted the number of vacuoles in 10 randomly chosen cells. If we did not find 10 complete cells in the field of view on the first slide, we then made another slide at the specified time and temperature until we could observe a total of 10 cells to get significant results. Most vacuoles were distinctly red and circular while others were oval-shaped (see Figure 2). They were contained within the cell membrane. Cells that had burst or had vacuoles outside of the cell membrane were not counted. We did count individual vacuoles that appeared clumped together in the centre of the cell. Statistical analysis was conducted using averages and 95% confidence intervals.

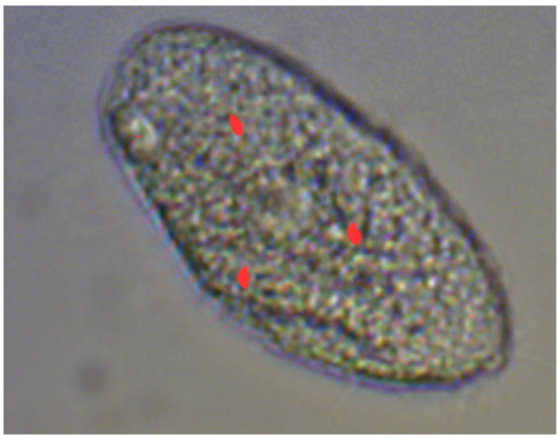

Figure 2. This picture indicates potential regions to find red vacuoles inside of a *T. thermophila* cell as well as the vacuole's potential size.

Results

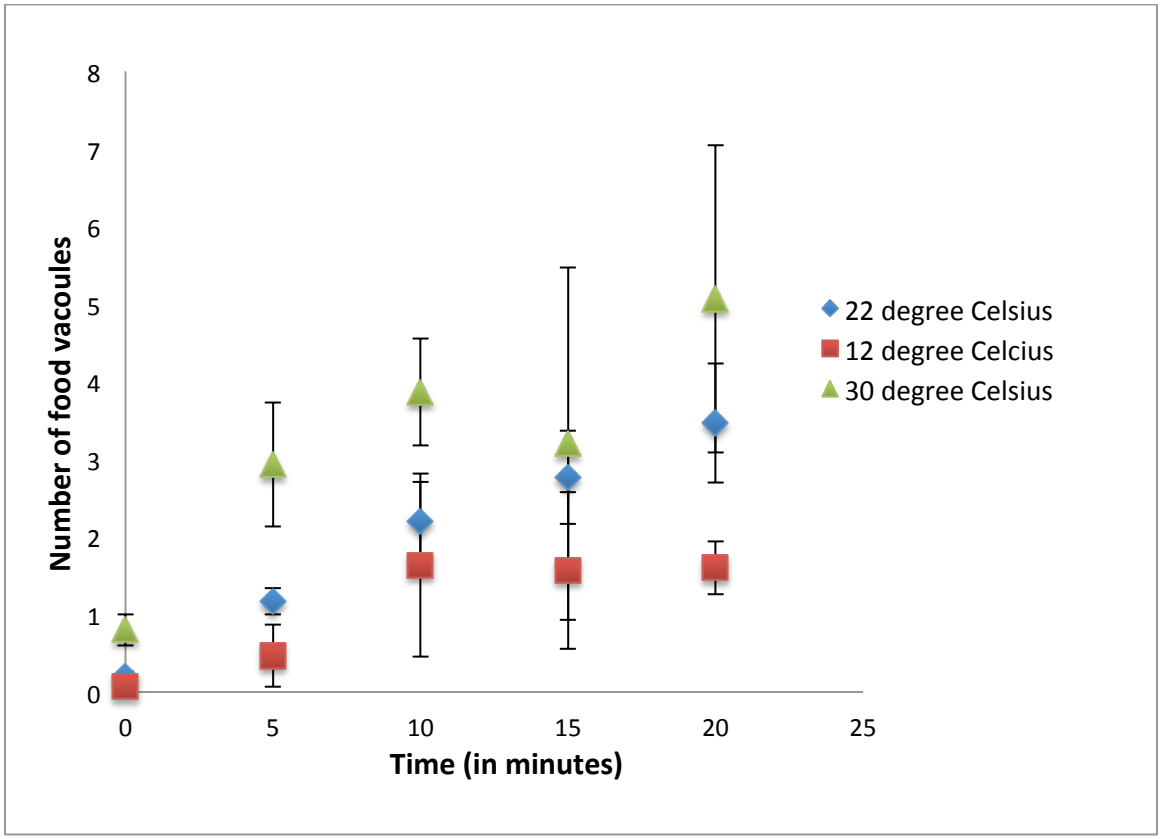

Figure3. The average number of food vacuoles was measured at a five minute interval for 20 minutes in 30 cells from three replicates incubated at 12° C, 22° C and 30° C, respectively. At 30° C, the average number of food vacuoles was significantly higher than those of the control and the 12° C treatment after 0, 5, and 10 minutes passed after adding the red *T. thermophila* medium, respectively. Moreover, the average number of food vacuoles at 30 ° C was significantly higher than that of the 12° C treatment at 20 minutes. Furthermore, the average number of food vacuoles at 22 ° C was significantly higher than that at 12 ° C at 5, 15, and 20 minutes. The error bar for each mean represents a 95% confidence interval.

In Figure 3, the average number of food vacuoles present in 30 *T. thermophila* cells from three replicates generally increased in both treatments and the control as time passed after adding red *T. thermophila* medium. At 30° C, the average number of food vacuoles increased almost linearly during the first 10 minutes, with a greater rate in the first 5 minutes than the following 5 minutes. However, the number of food vacuoles dropped by approximately 17% at 15 minutes. It rose again at 20 minute to a maximum number of food vacuoles in our observations in this treatment.

For the 22° C treatment, the average number of food vacuoles increased almost linearly at a lower rate than that at 30° C. The maximum number of food vacuoles was 3.5 in average and appeared at 20 minutes.

For the 12° C treatment, a peak of the number of food vacuoles occurred at 10 minutes. Food vacuoles in cells increased to the peak during the first 10 minutes, declined at 15 minutes and reached a plateau at 20 minutes.

Qualitative Observations

At 12° C, we observed one or two red food vacuoles outside a couple of cells and some irregular shaped cells. At 22° C, irregular shaped cells were also present. In addition, we found some lysed cells at 20 minutes and the red medium seemed to spread over the burst cells. At 30° C, a few cells had triangular or rod-like shapes and lysed at 20 minutes too. Moreover, one or more food vacuoles were present outside of some cells.

Sample Calculation

At 30° C and at time = 0 minutes:

1) Average number of food vacuoles present in three replicates (n=3) of 10 cells
= (1+0.7+0.7)/3=0.80 number of cells.

2) Standard deviation = square root of $\{[(1-0.8)^2+(0.7-0.8)^2+(0.7-0.8)^2]/(3-1)\}$
= 0.17320508 number of food vacuoles.

3) 95% Confidence Interval = mean +/- 1.96*(standard deviation / square root of sample size)
= 0.80+/-1.96*(0.17320508/square root of 3)
= [0.60, 1.00]

Discussion

The results of the experiment are statistically significant, allowing us to provide support for the alternate hypothesis and reject the null hypothesis. The results of the experiment are fairly consistent with the literature and similar experiments conducted by scientists in the past. One of

these past experiments was conducted by Nilsson (1977) who concluded that there are four primary stages in food vacuole formation in the *Tetrahymena* species. These stages include the growth of the vacuolar membrane, filling up of the vacuole, "closing off" of the vacuole, and the movement of the vacuole away from the cytostome. Excluding the last two stages, which are out of the scope of this experiment, this section will provide an explanation of the results with an emphasis on the stages of vacuole formation, discuss the consistency of the results with previous experiments, as well as provide possible reasons for variation and sources of error.

From Figure 3 it is clear that the effect of temperature does indeed increase food vacuole formation in *T. thermophila*. This is most evident in the first five minute interval, where the 95% confidence intervals for the mean number of food vacuoles do not overlap for any of the treatments. The number of food vacuoles increases from an average of 0.4 vacuoles per cell at 12° C to 1.2 vacuoles per cell at 22° C (room temperature) and to 2.8 vacuoles per cell at 30° C. These results may be explained considering the mechanism of vacuole membrane formation. Both Nilsson (1977) and Weidenbach (1974) agree that the formation of food vacuolar membrane occurs by an accumulation of membrane renewal vesicles at the cytostome of the *T. thermophila* cells. From studying the composition of lipids and proteins in the membranes, Weidenbach (1974) found that the vacuolar membranes arise from a vast pool of cytoplasmic membranes. Furthermore, considering that this stage of membrane formation only lasts seconds, (Nilsson 1977), an explanation for why numerous vacuoles were counted just 5 minutes after the introduction to particulate food material is provided. It is also important to note that in theory no food vacuoles could have formed in the 0 minute time interval but due to the few seconds time difference between adding and mixing the food dye and introducing a sample of cells to the prefer, some cells had already started to form food vacuoles. This provides even more evidence

that food vacuole formation requires only seconds to initiate. Of course, due to the temperature optimum of these cells lying around 28 to 30° C (Jacobs et al. 2006), it is obvious why the number of vacuoles tends to increase with the increase in temperature from 12° C, to 22° C, and to 30° C.

After the first five minute interval and completion of the first stage of vacuole formation, the number of vacuoles per cell tends to level off although still increasing a considerable amount. There are many plausible explanations for this levelling off, but the most compelling argument is that this is due to a depletion of limiting factors, such as vesicles for membrane formation or depletion of structural proteins and enzymes that aid the construction and maintenance of vacuoles (Weidenbach 1974). In a similar experiment, Nilsson (1977) stained vacuoles with a dye and observed digestion rates by recording the disappearance of stained vacuoles. It was concluded that the metabolism of food vacuoles requires a minimum of half an hour (as this was the minimum recorded duration of time for a stained food vacuole to disappear) although on average, it takes approximately two hours for the digestive cycle to complete. This finding has two important implications for the current experiment. First, since metabolism of the vacuoles take a minimum of half an hour, it can be safely assumed that none of the vacuoles that formed in the earlier time intervals could have possibly disappeared by the final 20 minutes as this would affect our count. In other words, it is safe to assume that exocytosis does not overlap with the process of phagocytosis in the experiment. If the duration of the experiment was longer than 30 minutes, this assumption would not be valid. Secondly, because vacuole formation declined after 30 minutes, it is evident that ingestion is fairly rapid at the beginning and declines shortly afterward. Again, we are confident that it is the phagocytosis of nutrients that is decreasing because exocytosis does not begin until a minimum of 30 minutes. Thus, it is clear why the rates

of vacuole formation tend to level off after the first five minute interval, although still retaining a difference in the number of vacuoles with the temperature differences with higher temperature corresponding to a greater number of average vacuoles per cell.

Two interesting observations were made during the experiment. First, it was noted that many cells in the 0 and 5 minute time intervals were lysed, or severely deformed. This may be explained by the fact that vacuoles in *T. thermophila* are continuous with the endoplasmic reticulum as well as an intricate network of nephridial tubules (Elliot and Bak 1964). Therefore, if too much stress is put on vacuoles, causing them to rupture, it would likely also complicate this connected network of ER and tubules leading to a lysed or a deformed cell. As a result, this was also a possible cause of error in our experiment, as the number of vacuoles in these deformed or lysed cells could not be counted and therefore, they were discarded. Second, a major biological variation was noted as some cells were particularly long and rod shaped. Again, Elliot and Bak (1964) suggest that this may be due to the fact that some cells with numerous vacuoles occasionally choose to organize their rough endoplasmic reticulum into a pattern of stacks to better organize their contents, and this pattern of stacks causes the cells to appear long and rod shaped.

Conclusion

The increasing temperature in which *T. thermophila* cells were incubated played a crucial role in their food vacuole formation. As the temperature increased to the organism's optimal growth temperature, at some time points, especially at 5 minutes passed after adding the red medium, food vacuoles increased correspondingly. This result is statistically significant and partially supports our hypothesis that states the metabolism increases as the temperature increases.

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