The Effect of Tonicity on Cell Volume and Phagocytosis in *Tetrahymena*

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Introduction

Background

Tetrahymena pyriformis, is an important single-celled freshwater eukaryotic model organism that is easy and inexpensive to maintain in a laboratory setting.
Tetrahymena pyriformis shares many biological features with some types of white blood cells (phagocytes) found in vertebrates and which are involved in immune defence against infections through their abilities to seek out, ingest and kill invading microorganisms.
Phagocytosis (cell eating) is a process by which cells engulf particles from their environments. It is used by *Tetrahymena* to ingests food particles which are later digested in their cells.
In mammals, various abnormalities in fluid and electrolyte balance are associated with changes in cell volume, impaired phagocytosis, enhanced susceptibility to infections and, in extreme situations, cell and/or organism death.

Purpose

This study examines the effects of environmental changes in water and solute content on cell structure, cell volume and phagocytosis using the model organisms *Tetrahymena pyriformis*.

Methods and Materials

Cells

Tetrahymena pyriformis were obtained from Carolina Biological Supply Company and cultured in proteose peptone media (Carolina, NC).

Cell cultures

To achieve dilute (hypotonic) environmental conditions, cells in culture media were mixed with distilled water, using ratios between 1:1 and 1:6, and left for periods between one hour and 24 hours in 24 well culture chambers (Costar, Sigma-Aldrich).
To achieve more concentrated (hypertonic) environmental conditions, cells were exposed to Mannitol (Sigma-Aldrich, ON), a carbohydrate which does not enter cells, at either 12.5 mM, 25 mM or 50mM, for periods between one hour and 24 hours in 24-well culture chambers.

Phagocytosis assay

To assess phagocytosis, a 1% India ink solution (Merlan Scientific, ON) was made using distilled water and added to cell cultures for 30 minutes (1). Thereafter, cells were fixed with 3% glutaraldehyde (Fisher Scientific, ON) and the number of India ink vacuoles in each of at least 20 random cells was assessed using a compound light microscope. From these counts, the mean number of India ink containing vacuoles was determined under different cell culture conditions.

Microscopy

A Nikon compound light photomicroscope (Nikon Eclipse 50i photomicroscope) was used to visualize cells and to count India ink vacuoles in their cytoplasm.

Results

Tetrahymena in Different Media:

Fig 1. *Tetrahymena* cultured in proteose peptone medium (control) for 24h and viewed using 100X objective lens.

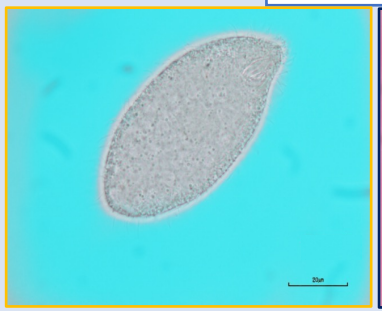


Fig 2. *Tetrahymena* in a medium diluted with distilled water at a ratio of 1:6 (hypotonic conditions) and cultured for 24h and viewed using 100X objective lens..

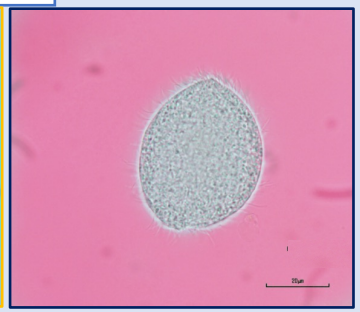


Fig 3. *Tetrahymena* cultured in Mannitol 50mM (hypertonic conditions) for 24h and viewed using 100X objective lens..

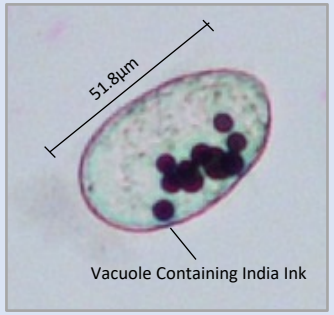
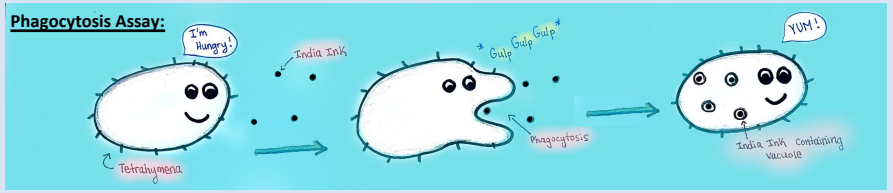


Fig 4. *Tetrahymena* cultured with 1% India ink for 30 min and viewed using 10X objective lens.

Phagocytosis Assay:



Tetrahymena Cell Volume:

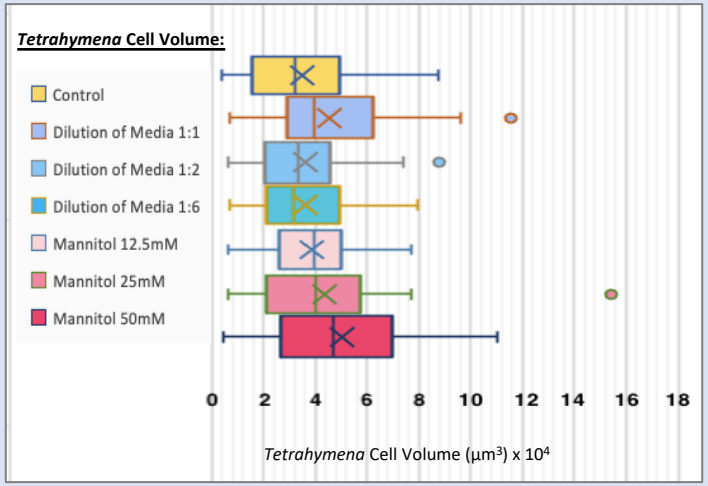


Fig 5. *Tetrahymena* cell volumes following 24h cell culture in proteose peptone medium (control), dilutions of medium with distilled water (hypotonic conditions) or medium with added Mannitol (hypertonic conditions), presented as box and whisker plots.

Tetrahymena Phagocytosis:

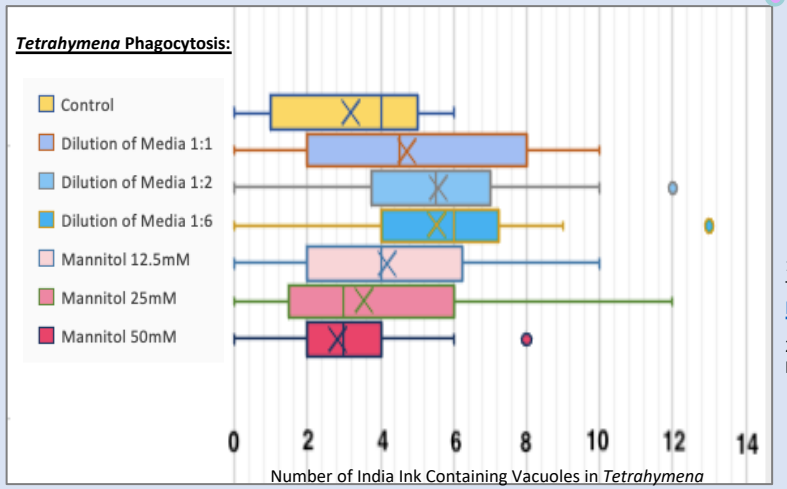


Fig 6. *Tetrahymena* phagocytosis of 1% India ink presented to cells over 30 min and following 24h cell culture in proteose peptone medium (control), dilutions of medium with distilled water (hypotonic conditions) or medium with added Mannitol (hypertonic conditions), presented as box and whisker plots.

Cell volume determination

Tetrahymena cell width and length were measured from photomicrographs using the scale bar and photomagnification of the images. A random sample of cells (30-50) were assessed. Cell volume was quantified based on the assumption that Tetrahymena are symmetrical on their long axis. Therefore, cell volume (V) was determined through the following equation (L. Millis):

$V = 1/6 \pi * W^2 * L$

Statistical analysis

Statistical significance was analyzed through one-way analysis of variance and subsequently determined through a Tukey HSD test. $p<0.05$ was considered significant (2).

Conclusions

1. *Tetrahymena* are ciliated unicellular protists and easily visible by light microscopy without staining. The cells vary in size and volume with a median volume of 34749.17 μm^3 in proteose peptone culture medium.
2. *Tetrahymena* controlled cell volume effectively in hypotonic conditions, including a culture medium: distilled water dilution of 1:6. Beyond this level their regulatory capacity was exceeded, and cells burst (data not shown).
3. Hypertonic conditions (with mannitol added to culture media) reduced *Tetrahymena* cell volume in a concentration-dependent manner, but this did not reach statistical significance.
4. As tonicity increased, phagocytosis tended to decrease, with change most notable in Mannitol 50mM solutions (See figure 6) . This was statistically significant for one of the experiments conducted (data not shown).

Future experiments

Future experiments using larger sample sizes and longer culture periods in either hypotonic or hypertonic solutions, will likely be needed to support these preliminary findings.
Additional studies should examine mammalian phagocytes to provide deeper insight into how tonicity affects cell volume and phagocytosis regulation in multicellular eukaryotes. These studies may facilitate greater understanding of how fluid and electrolyte imbalances may contribute to phagocyte dysfunction.

References

1. Bozzone, D. (2000). "Investigating Phagocytosis in Tetrahymena," *The American Biology Teacher* 62(2), 136-139 https://doi.org/10.1662/0002-7685(2000)062[0136:IPIT]2.0.CO;2

2. Lowry, R. (n.d.). *Statistical computation web site*. VassarStats. Retrieved April 4, 2022, from http://vassarstats.net/

Acknowledgments

Utmost gratitude to Lynette Sigola for giving me all assets needed to complete this project, as well as aiding in editing. Much appreciation to Len Millis for providing the formula for the cell volume calculations and to Bruna Silvestroni Pimentel for teaching me how to passage the Tetrahymena cultures.