An Investigative Approach to the Study of Phagocytosis in *Tetrahymena*

Donna Bozzone

Department of Biology, St. Michael's College, Colchester, VT, USA, 05439
(802) 654-2627 dbozzone@smcvt.edu

Tetrahymena, a ciliated protozoan, has many attributes that make it an ideal organism for the students' study of cells. *Tetrahymena* can be obtained easily, grow in very simple media, and are readily used to explore ciliary motion, organelle structure and function, cell behaviors, and cell morphology. Because of the ease with which *Tetrahymena* can be grown and manipulated, they are especially suited for use in inquiry-based labs and student projects. The specific focus of this workshop was to examine the processes of phagocytosis and vacuole formation in *Tetrahymena*. The effects of several factors on these phenomena were studied.

Although the cells we used for this workshop were grown in a bacteria-free medium, *Tetrahymena* do eat bacteria or other small cells, and organic debris by the process of phagocytosis. When a hungry *Tetrahymena* encounters food, it uses its cilia to sweep the food into the cell's oral groove. This process can be visualized by feeding stained yeast cells or India Ink (!; Keenan, 1984) to *Tetrahymena*. We used the India Ink protocol to study this process. One simply "feeds" a dilute suspension of India Ink to *Tetrahymena*, and quantitates the number of black vacuoles, which form in a defined time period.

Using this simple procedure, many interesting questions can be addressed regarding phagocytosis and vacuole formation. Examples of testable questions generated by my students (and me) include:

1. Does the concentration of India Ink affect the rate of vacuole formation?
2. Does the nutritional state of *Tetrahymena* affect the rate at which food vacuoles form?
3. Is the cytoskeleton involved in the formation of vacuoles? If so, are the microfilaments important? Microtubules? Both?
4. Will *Tetrahymena* select specific items for phagocytosis when presented with mixtures of food?
5. Will the rate of phagocytosis vary with temperature?
6. Will the velocity with which *Tetrahymena* cultures are agitated affect the rate of phagocytosis?
7. Will deciliated cells eat?

In our workshop, we focused our attention on questions one and two from the above list but I have also provided the protocol for examining question three as well as some sample data for questions 1-3.

Procedures

The following procedure for quantitating vacuole formation by feeding India Ink to *Tetrahymena* is a slight modification of the technique described by Katherine Keenan in Volume 4

of the ABLE Proceedings (you see it pays to look at those back volumes….). The protocol is written so that it could be given to students as instructions.

Does the Concentration of India Ink Affect the Rate of Vacuole Formation?

1. *Tetrahymena* grown in 2% proteose peptone for 48-72 hours will be provided.
2. Prepare three test tubes as follows:
   3 ml *Tetrahymena* + 3 ml 1% ink
   3 ml *Tetrahymena* + 3 ml 5% ink
   3 ml *Tetrahymena* + 3 ml 10% ink

3. Observe the behaviors of cells from each tube. Record your observations.
4. Sample tubes at 0, 2, 5, 10, 20, and 30 minutes. To sample, pipette 20 microliters of cells in the ink suspension into a microfuge tube containing 10 microliters of 3% glutaraldehyde.
5. Count the number of vacuoles per cell for at least 20 cells for each time point.
6. Prepare a table and graph to display your results.

Does the Nutritional State of Tetrahymena Affect the Rate at Which Food Vacuoles Form?

1. You will be provided with *Tetrahymena* which have been growing for 48-72 hours in 2% proteose peptone, and *Tetrahymena* which were grow for 48-72 hours but then starved for at least 24 hours.
2. Prepare two test tubes as follows:
   3 ml well-fed *Tetrahymena* + 3 ml 1% ink
   3 ml starved *Tetrahymena* + 3ml 1% ink
3. Observe the behaviors of cells from each tube. Record your observations.
4. Sample cells from each tube at 5, 10, 20, and 30 minutes and quantitate vacuole formation as described above.
5. Prepare appropriate tables and figures to display your results.

Is the Cytoskeleton Involved in the Formation of Vacuoles?

A. The Effect of Cytochalasin B (an inhibitor of microfilaments).
   1. Add 2 ml of *Tetrahymena* and to a tube containing 20 microliters of cytochalasin B (5 mg/ml).
   2. Incubate the cells with cytochalasin B for 10 minutes.
   3. After this incubation, add 2 ml of 1% ink to the tube.
   4. Observe and record the behaviors of the cells.
   5. Sample every 10 minutes for 30 minutes and quantitate vacuole formation as described above.
   6. Spin down the cells (3 or 4 minutes at 1000 g) and resuspend the pellet in 2% proteose peptone. Spin and resuspend the cells two more times. Resuspend the final pellet in two ml of proteose peptone. (An alternative procedure is to incubate cells in cytochalasin B or colchicine for 30 minutes, and then wash them.)
   7. Add 2 ml of 1% ink to the washed cell suspension.
   8. Observe and record cell behaviors.
   9. Sample for 20 minutes and quantitate vacuole formation as described above.
   10. Prepare appropriate tables and graphs to display your results.

B. Effect of Colchicine (an inhibitor of microtubules)

Follow the procedure detailed for examining the effect of cytochalasin B, except use colchicine (400 mg/ml).

Sample Data

Table 1. Phagocytosis of Different Concentrations of India Ink By *Tetrahymena*

Time (min)	mean number of vacuoles per cell 1 % Ink	5 % Ink	10 % Ink
0	2.1	1.5	0.4
2	3.8	2.6	1.1
5	4.6	3.5	1.3
10	4.8	4	1.4
20	6.1	4.8	1.5
30	7.1	4.5	2.4

Table 2. Phagocytosis in Starved and Well-fed *Tetrahymena*

Time (min)	mean number of vacuoles per cell Starved cells 1 % Ink	5 % Ink	Well-fed cells 1 % Ink	5 % Ink
0	1	0.2	1.9	1.6
5	2	0.9	4.4	3.6
10	3	1	5.9	4.9
20	3.8	1	7.2	6
30	4	1.4	8.3	5.3

Table 3. Effects of Cytochalasin B and Colchicine on Phagocytosis in *Tetrahymena* (and Recovery)

Time (min)	mean number of vacuoles per cell Control	Cytochalasin B	Colchicine
5	2.7	0.9	2
10	4.6	0.95	3.4
20	6.4	0.95	4.95
Washed samples			
10	3.1	1.7	2.5
20	5.9	2.95	5.3

Recipes

2% proteose peptone: 2g-proteose peptone per 100 ml distilled or deionized water. Autoclave 20 minutes slow exhaust. For reasons I am not sure of, this medium does not store well; the cells stop growing in it. I use it within a week of making it.

To culture *Tetrahymena*, inoculate 1 ml into 25 ml of proteose peptone in a 125 ml Erlenmeyer flask (the ratio of medium volume to total flask volume should be 1:5 or lower). Incubation at room temperature, 20-22 degrees centigrade is best. *Tetrahymena* can be purchased from many suppliers (I use Carolina Biological). Be sure to order the "bacteria-free" cultures.

Salt solution for starving Tetrahymena: 6 mg KCl, 4 mg $CaHPO_4$ (or $CaCl_2$), 2 mg $MgSO_4 -7H_20$ per liter of distilled or deionized water. Autoclave 20 minutes, slow exhaust.

To starve *Tetrahymena*, spin down cells from a 24-72 h culture, and resuspend the pellet in salt solution. Centrifuge and wash cells two more times. Resuspend the final pellet in a volume of salt solution equal to the volume of the medium you initially removed. The resuspended cells should be incubated at room temperature.

Ink: As Keenan (1984) described, the type of ink you use matters a great deal because some have detergent in them (these won't work). I use Hunt-Speedball. All dilutions are made in distilled or deionized water.

Cytochalasin B: 5 mg/ml in 70% ethanol makes a very good stock solution. We use it at a final concentration of 50 ug/ml. I suspect lower concentrations might work but have not tested this yet.

Colchicine: 400 mg/ml in distilled or deionized water. We use it at a final concentration of 4 mg/ml, and as with cytochalasin, I have not tested lower concentrations yet.

Acknowledgement

This work was supported in part by NSF-ILI grant # 9205174.

References

Hoffmann, E.K., L. Rasmussen, and E. Zeuthen. 1974. Cytochalasin B: Aspects of phagocytosis in nutrient uptake in *Tetrahymena*. Journal of Cell Science, 15: 403-406.

Keenan, K. 1984. Uptake of particles by the ciliate *Tetrahymena*. In: Harris, C.L. (ed.) Tested Studies for Laboratory Teaching. Proceedings of the Fourth Workshop/Conference of the Association for Biology Laboratory Education (ABLE).

Nilsson, J.R., T.R. Ricketts, and E. Zeuthen. 1973. Effects of cytochalasin B on cell division and vacuole formation in *Tetrahymena pyriformis* GL. Experimental Cell Research, 79: 456-459.

Ricketts, T.R. 1972. The induction of endocytosis in starved *Tetrahymena pyriformis*. Journal of Protozoology, 19: 373-375.

Reprinted From: Bozzone, D. 1998. An investigative approach to the study of phagocytosis in *Tetrahymena*. Pages 347-350, *in* Tested studies for laboratory teaching, Volume 19 (S. J. Karcher, Editor). Proceedings of the 19th Workshop/Conference of the Association for Biology Laboratory Education (ABLE), 365 pages.