Phagocytosis and Vacuole Formation in *Tetrahymena*

TEACHER’S MANUAL WITH STUDENT INSTRUCTIONS
Phagocytosis and Vacuole Formation in *Tetrahymena*

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Phagocytosis and Vacuole Formation in *Tetrahymena*

The study of phagocytosis and vacuole formation in *Tetrahymena* is a rich research topic and versatile teaching tool. The instructions for the experiments presented here can be adapted for a variety of teaching needs, from introductory to advanced. Moreover, if appropriate for your class, this is also an excellent system for student projects and independent work.

The experiments are designed for students to work in groups of 2 to 4. Depending on the particular experiment, one group of students could complete the entire experiment in a lab period. In some cases, you might want to have the entire class do a single experiment and assign parts to individual groups.

**Materials**

*Included in the kit*
- axenic *Tetrahymena*
- slides
- coverslips
- pipets
- India ink
- *Tetrahymena* medium (proteose peptone)
- Centrifuge tubes, 15 mL
- Protoslo®
- Lugol solution

*Needed, but not provided*
- test tubes or small vials, for holding mixtures of ink and cultures
- flasks
- autoclave or pressure cooker
- compound microscopes
- incubator(s)
- refrigerator or ice
- centrifuge
- Salt solution (for starvation)

*Optional:*
- glutaraldehyde
- ethanol
- carmine
- carbon particles
- Erlenmeyer flasks
General Methods and Procedures

Culturing *Tetrahymena*

It is easy to grow *Tetrahymena* for your class. To maintain your stock culture, inoculate 25 mL of fresh medium with 1 mL of a growing *Tetrahymena* culture once a week. Incubate the cultures at room temperature (20–22°C). It is fine to incubate at lower temperatures, although cell population growth will slow. It is also safe to incubate at a higher temperature; in this case, the cell population growth rate will increase. Don't exceed 29°C for stock culture incubation. Each team of 2–4 students can work from one culture. Students do not need to maintain aseptic technique when working with their cultures since you have the “clean” stock culture. *Tetrahymena* can be cultured in either tubes or flasks, but growth is better in flasks, where diffusion of oxygen is greater.

Fixation Methods

Lugol solution is an excellent fixative, but if you wish to see fixed cells without staining the cytoplasm, a drop of 1% formaldehyde, 1% glutaraldehyde or 95% ethanol added to a couple of drops of cells will do the job. You should be aware that the ethanol will eventually evaporate from a microscope slide but not before you can get some very good observations. **Note:** If you wanted to observe fixed cells that had eaten carmine, you would need to use glutaraldehyde or ethanol as your fixative because the staining from Lugol solution obscures observation of the red vacuoles.

India Ink

The brand of India ink used for phagocytosis matters because some companies put detergent in their inks. Detergent lyses cells. The ink present in this kit contains no detergent. It is supplied as a 10% stock so you need to dilute it for use. India ink in the bottle, from an art store, is considered 100%. A possible substitute for using India ink is to make a suspension of carbon particles or carmine particles and feed it to cells. **Note:** If you want to see what happens to neutral red staining when cells are feeding, you must use India ink or a carbon particle suspension in order to see the red staining. Since carmine is red, it is not suitable for this exercise.
Recipes

**Proteose peptone medium (as supplied in the kit)**
- 2.5 g proteose peptone
- 2.5 g tryptone
- 0.1 g K₂HPO₄
- 500 mL distilled water

Adjust to pH 7.2. Place 25 mL into each of 20 125-mL Erlenmeyer flasks. Plug the tops of the flasks with cotton or a foam plug. Cover the tops with aluminum foil. Autoclave 20 minutes slow exhaust (liquid cycle). This is the medium supplied in the kit.

**2% Proteose peptone (alternative culture medium)**
- 10 g proteose peptone
- 500 mL distilled water

Place 25 mL in each of 20 125-mL Erlenmeyer flasks. Plug the tops of the flasks with cotton or a foam plug. Cover the tops with aluminum foil. Autoclave 20 minutes at slow exhaust (liquid cycle).

After cooling, this medium should be refrigerated and used within 2 months. You can inoculate *Tetrahymena* directly into medium taken out of the refrigerator. Growth is more luxurious in this medium than in the one detailed above.

**Dilute Salt Solution (for starvation)**
- 6 mg KCl
- 4 mg CaHPO₄ (or CaCl₂)
- 2 mg MgSO₄•7H₂O
- 1 L of distilled water

Plug and cover the top of the flask. Autoclave 20 minutes at slow exhaust (liquid cycle). Cool to room temperature before use. This solution can be stored at room temperature.

**Preparation**

Copy the Student Instructions. Each student (or student group if they work in teams) will need a culture of *Tetrahymena* or they can start their own cultures as part of the lab work.

**References**

