In Figure 1 you can see our results from centrifugation

# Phagocytosis of Paramecium by Amoeba Proteus

## An Idependent Research Project

**Samara Audet**
**Wheaton College**
**Norton, MA 02766**
**Lab Partner - Mike Smith**

## Introduction

The Amoeba proteus is a unicellular organism. It has the ability to extend its cytoplasm by means of its pseudopodia. These pseudopodia are used to feed. They can envelope the food source with its' plasma membrane and take it within the cytoplasm in the form of a vacuole or cavity (Encarta, 2004). The digestive enzyme located in the vacuole or cavity can break down the food and cause it to become a soluble chemical substance (Encarta, 2004). These soluble chemicals will then be transported into the cytoplasm for the Amoeba proteus molecular usage (Encarta, 2004). Ultimately, the broken down food source will be secreted in the Amoeba proteus through exocytosis (Encarta, 2004). The Hoescht dye that we will be using is known for its ability to stain DNA by becoming flourescent. It can bind to DNA in cells and has the ability to penetrate both living and fixed cells. The Hoescht dye will form nonconvalent bonds with base pairs of the DNA without distorting the DNA helix (Everything.com, 2004). It is commonly used for determining the location of DNA in the nucleus of cells (Professor Robert Morris, personal communication, October 2005).

The original idea of this experiment was to label the DNA present inside paramecium bursaria nuclei with Hoescht dye and feed it to the protozoan Amoeba proteus. This was in hopes that the stained blue dye (due to the Hoescht) would be visible inside the protozoan after the protozoan had phagocytosed. We were interested in finding the location, such as the nucleus or cytoplasm, of the paramecium DNA in the Amoeba proteus. Our original hypothesis was that the majority about 90% of the ingested, stained paramecium DNA would be found in the cytoplasm of the Amoeba proteus. This would be apparent right after the phagocytosis occured. We also hypothesized that the paramecium could be stained with Hoescht dye alive and presented to the Amoeba proteus on a slide.

Another hypothesis was in regards to the procedure that we planned to carry out. In this method we conjectured that approximately 2mL of paramecium in pond water would survive up to 15 minutes after one minute of centrifugation on setting one. This would ultimately help us concentrate the paramecium in order to present them to Amoeba proteus. The interest of this study was to learn how a living single-celled organism, Amoeba proteus, ingested food and where in the cell it was moleculary broken down. We wanted to know where the food source proved to be helpful in supporting the organism. The idea of endocytosis by the Amoeba proteus and the process of taking and breaking down the DNA of its food was an interesting mechanism to us.

We also looked to test the amount of pseudopodia present in the Amoeba proteus depending on the food source available in their current environment. In this experiment, we predicted that the greater the presence of a food source for the Amoeba protues, than the greater the amount of pseudopodia present on the organism. Also, we hypothesized that the Amoeba proteus pseudopodia would react more in the presence of yeast with an increase in pseudopodia. We wanted to inquire if a food source triggered these plasma extension in a search for food.

In this study, we introduced Hoescht stained paramecium in the presence of Amoeba proteus hoping to witness phagocytosis by the Amoeba. We also observed Amoeba proteus in an environment lacking paramecium, filled with paramecium, and the presence of yeast in order to determine the amount of pseudopodia present depending on the food source and amount.

## Materials and Methods

- Paramecium Bursaria (in pond water)
- Amoeba Proteus (in pond water)
- Peacock Pond Water
- Coverslips
- Glass Slides
- Gemini SpotInsight (ae) Nikon Eclipse E200 microscope
- Capricorn SpotInsight (ae) Nikon Eclipse E200 microscope
- Dissecting Microscope (Nikon 102)

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- Pasture Pipettes
- Plastic Pipettes
- Tweezers
- Coverslip chips
- International Clinical Centrifuge
- 50mL Centrifuge Tubes
- Hoechst dye
- Tin Foil
- Rapid Rise Highly Active Yeast
- KimWipes

The first step towards conducting the experiment involved viewing the Amoeba proteus under a dissecting microscope and making sure we could see them and capture them. This served as the control for the phagocytosis experiment. The container of Amoeba proteus was placed on the dissection microscope (Nikon 102) and the contents were viewed on setting five. This allowed for the Amoeba proteus to be seen. A glass pasture pipette was used to remove the Amoeba proteus one by one as they were placed on respective slides. Once the Amoeba proteus was placed on the slide, cover slip chips were placed in a circle around the specimen with tweezers in order to keep the coverslip from crushing the Amoeba to death. Then on the Gemini Spot Insight (ae) Nikon Eclipse E200 microscope the Amoeba proteus could be viewed under three obective lens including 40x, 100x, and 400x.

Next paramecium were centrifuged. Approximately 10mL of paramecium were placed in a 50mL centrifuge tube. The centrifuge tube was then placed in the International Clinical Centrifuge for one minute at setting one. Then a single drop from the bottom pellet of paramecium in the centrifuge tube was viewed with the Gemini compound microscope and the results were recorded. The same process was then repeated except centrifuging the paramecium for two minutes on setting one. Those results were then recorded. The control group was the container the paramecium arrived in.

Then the final method to conduct the phagocytosis experiment was carried out. First, the paramecium were viewed underneath the dissecting microscope to make sure they were alive and well. Then 2mL of paramecium (in the pond water) were placed in a 50mL centrifuge tube. Next 2mL of peacock pond water was placed in another 50mL centrifuge tube. Then 200 microliters of Hoescht dye was added to the 2mL of peacock pond water. One unit of Hoescht in stock will equal ten units in the total Hoescht solution. The ratio will be 1:2000. Therefore 200 microliters were added to the 2mL solution of peacock pond water (Professor Robert Morris, personal communication, November 2005). That centrifuge tube was then agitated. Next, the peacock pond water with Hoescht dye and 2mL of paramecium in pond water wer combined into one centrifuge tube. The centrifuge tube was then centrifuged with the International Clinical Centrifuge for 45 seconds at setting one. The Nixon Eclipse E400 microscope at the capricorn computer was then used to view the stained paramecium. Next, ten Ameoba proteus were added to the solution of paramecium, Hoescht dye, and pond water. That centrifuge tube was then centrifuged for 30 seconds. Finally, the Ameoba proteus were imaged for 30-45 minutes.

The second effort to conduct this procedure was then altered a bit. The amount of paramecium in pond water and peacock pond water were increased to 8mL respectively. Therefore the amount of Hoescht dye added to the peacock pond water solution was increased to 400 microliters. The following steps remained the same up until the addition of Amoeba proteus. Instead, single amoebas were obtained using pasture pipettes and the dissecting microscope and were placed on their own seperate slides. Then the stained and centrifuged paramecium were taken up by a plastic pipette and a single drop was placed on each slide containing one ameoba. The rest of the experiment was conducted in a similar manner.

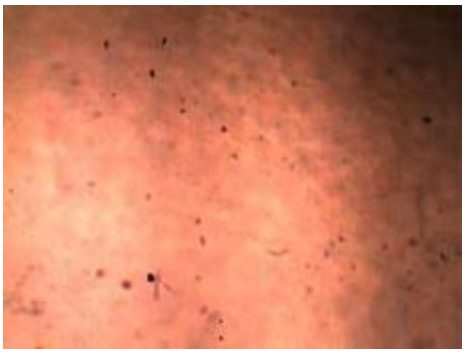
Also six empty slides and six coverslips were obtained. Coverslip chips were then placed on all six slides in order to prevent crushing of the specimen by the coverslip. Then using the same technique of a pasture pipette and dissecting microscope single amoebas were placed on each slide. Next, a yeast solution was developed by adding .5g of Fleishmann's Rapid Rise Highly Active yeast to 20mL of peacock pond water in a centrifuge tube. The centrifuge tube was then shaken. A coverslip was then placed on the first two slides of single amoeba that were not obtaining any exra food source as a control group. The results were then recorded using a compound microscope. The next two slides of amoeba received a drop of paramecium before the coverslip was placed on the slide. Those results were recorded using a compound microscope. In the final step, two slides with amoeba had coverslips placed on them. The yeast solution was then added to the edge of the coverslip. The excess pond water was wicked off with a KimWipe. The results were then recorded with a compound microscope.

The analysis and quantification in this experiment involved the number of Amoeba proteus that phagocytosed on the slides (Mike Smith, personal communication, November 2005).

# Results

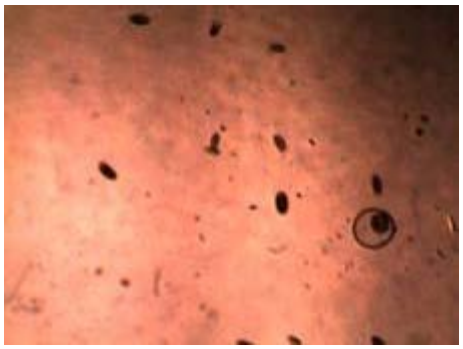
In Figure 1 you can see our results from centrifugation

Figure 1.1



Non-Centrifuged Solution - 40x Nikon Eclipse E200

Very few paramecium were seen lower density, upper portion of the centrifuge tube. Zero paramecium are present under the microscope.



Centrifuged Pellet - 40x Nikon Eclipse E200

This is from the pellet after centrifugation. Several paramecium were observed. Ten paramecium are completely present under the microscope. Two of the paramecium appear to be dead.

In Figure 1.1 you can see our results from the first centrifugation. The object of this experiment was to test the efficiency of centrifugation with the goal of concentrating the paramecium into a pellet at the bottom of the 50mL centrifugation tube. This in turn would make it easier to obtain a large amount of paramecium to introduce to the amoeba proteus. We centrifuged for about one minute on the lowest setting (setting one). This result supported our hypothesis of centrifugation (Mike Smith, personal communication, November 2005).

Figure 2.1

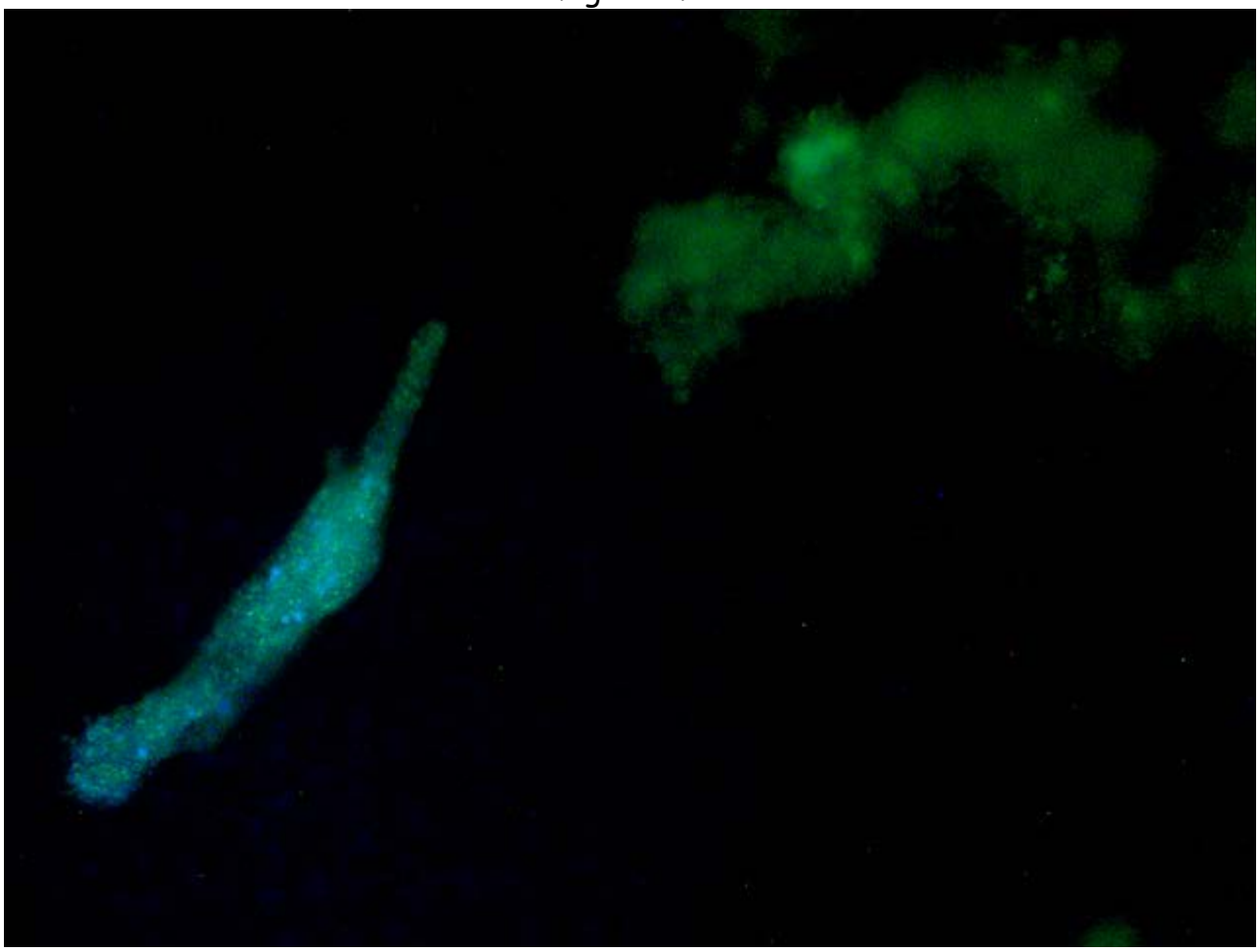


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Figure 2.2

In figure 2.1 and figure 2.2 the Amoeba proteus are present on the Capricorn Nikon Eclipse E400 microscope at 10x objective lens. The microscope is set so that flourescence is seen. The Amoeba proteus are clearly visible and the Hoescht dye indicates that the amoeba is multinucleated. These images do not support our hypothesis of phagocytosis of paramecium in Amoeba proteus (Mike Smith, personal communication, November 2005).

## Phagocytosis of Stained Paramecium by Amoeba proteus Imaged on the Capricorn Nikon Eclipse E400

| Trial One | Trial Two |
|---|---|
| 0 | 0 |

Table 1.1 quantitatively represents that no Amoeba proteus could be captured phagocytosing a paramecium with stained DNA. We were unable to test our hypothesis due to this outcome.

Table 1.1 The Amount of Pseudopodia Present in Differing Food Source

| No Extra Food Source | Presence of Amoeba | Presence of Yeast |
|---|---|---|
| 3 | 0 | 0 |
| 1 | 1 | 0 |

In Table 1.2 we observed that there appeared to be no real pattern to the amount of pseudopodia present in different food sources. The pseudopodia were defined as a prominent extension from the central or base area of the Amoeba protues. The

experiment in the presence of yeast failed to gain any results and we were unable to see amoeba proteus in one of the paramecium trials and both of the yeast trials. These data do not support our hypothesis of an increase in pseudopdia due to the presence of an additional food source. We predicted a greater number of pseudopodia in the presence of yeast and these data fail to back our hypothesis up.

# Discussion

In conclusion, we were unable to test our original hypothesis due to our inability to image phagocytosis of stained paramecium by Amoeba proteus. However, our methods of staining with Hoescht dye worked to our advantage. We were successful in staining live paramecium DNA with Hoescht dye and observing it underneath the microscope. The amount of Hoescht dye that was used may have been too much seeing as the Amoeba proteus DNA became stained as well (Figure 2.1 and Figure 2.2). We were unable to determine the amount of paramecium DNA present and the location of the DNA in an Amoeba after phagocytosis. The obtained image of Amoeba proteus stained with Hoescht dye provides a good example of how Amoeba proteus is multinucleated. This could not be detected as easily unless it was under flourescence.

Also, our method of centrifuging 2mL of paramecium in order to obtain a more concentrated solution of the organism worked to our benefit. The paramecium could survive the centrifugation seeing as only about 2% of the population died after that method took place (Figure 1.1). This method could be helpful in future experiments.

There was inconclusive data gathered to support the hypothesis of the Amoeba proteus pseudopodia increasing in number as the food source increased or in relation to the food type. The Amoeba proteus had three pseudopodia present in the first trial with no extra food source and one in the second trial with no extra food source. In the first trial of the Amoeba proteus with the extra paramecium food source no amoebs could be detected on the slide. In the seond trail one large pseudopod was present. The yeast experiment failed to gain any results because the concentration of yeast was too large at .5g of yeast to 20mL of peacock pond water. No definite conclusions could be found since the method failed to meet our expectations. In following experiments, I would plan to have more trials in each food source and bring the concentration of yeast down to .1g of yeast to 20mL of peacock pond water.

The main success of the experiment was our hypothesis about the methods and procedures that proved to be very helpful and succesful. We hypothesized that the method of centrifuging live paramecium for one minute on the lowest setting would obtain a greater concentration of paramecium at the bottom of the centrifuge tube. The data support that hypothesis (Figure 1.1). This method made working with paramecium easier since they were more concentrated after centrifugation. Also our supported hypothesis of staining paramecium at a 1:2000 ratio and having them survive with the ability to be seen under a microscope worked to our benefit (Professor Robert Morris, personal communication, November 2005). This was the first time that live specimen were seen with a flourescence microscope in the ICUC. In future experiments, I would plan to leave the stained paramecium in the presence of the Amoeba proteus for a longer period of time. This may allow for phagocytosis to take place. Also, a lower concentration of Hoescht dye may be used as to not stain the Amoeba proteus nuclei in the process.

My conjecture to why phagocytosis could not be observed and the lack of evidence to support any increase in pseudopodia amount involves an already present food source in the original container of Amoeba proteus. They were already well fed in their current environment. At the cellular level, the Amoeba proteus had sufficient amino acids present in their surroundings and no need to ingest the amino acids of the paramecium DNA. This would cause the Amoeba proteus to be uncooperative in our study.

# References Cited

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