

# The Effect of Nocodazole Treatment on the Pseudopodial Number in *Amoeba proteus*

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## Introduction:

Amoebae have extensions known as pseudopodia, which vary depending on intracellular streaming. Pseudopodia are important for amoeboid movement, which is the type of locomotion that amoebae employ. When an attractive source is near an amoeba, it will extend its pseudopods in the direction of the source in order to move closer (Ueda, 1994). The pseudopodia extend due to cooperation between actin polymerization and microtubule formation.

This system between the microtubules and the actin filaments enables migration, polarity and division. The microtubules extend from the cell body and interact with actin sites (Schober, 2007). Microtubules are hollow rods made up of tubulin dimers that can undergo cycles of growth and depolymerization, known as dynamic instability (Cooper, 2007). The microtubules of a cell enable transportation of vesicles as well as organization of the cell itself (Vasquez, 2007). Motor proteins, such as kinesin and dynein, travel along the microtubules carrying vesicle or other molecules (Cooper, 2007). Nocodazole is a common microtubule drug that causes rapid depolymerization. Lower concentrations of nocodazole will only affect the polymerization of tubulin, but at a high concentration, nocodazole can prevent a cell from undergoing mitosis (Vasquez, 2007).

This experiment is significant to investigate because it can determine how the depolymerization of microtubules affects a certain organism and if any other processes are in turn affected. *Amoebae proteus* is the system in which this experiment is conducted because these organisms are visible with the naked eye and cellular movements can easily be seen using light microscopy. *Amoeba proteus* is easily maintained and allows experiments to be conducted in vivo. These results would show how the microtubule system is connected to other systems in the cell. By looking at pseudopodia number, it can be determined if the microtubules in the amoebae are strongly affected by the nocodazole. In this study, amoebae are tested to determine if fewer pseudopodia form when the amoebae are treated with the drug, nocodazole, which inhibits the formation of microtubules. In order to test this hypothesis, still frames of a control amoeba and an amoeba treated with nocodazole will be taken for two minutes. The number of pseudopodia for the control amoeba and the experimental amoebae will be average for comparison.

## Materials and Methods:

Two flow cells were prepared in order to conduct the experiment. In order to prepare these flow cells, coverslip chips were arranged on two slides before the sample was given. *Amoebae proteus* were taken from a solution of pond water and placed onto the slide in the center of the coverslip chips. A coverslip was placed on top of the coverslip chips and the sample. VALAP was used on two parallel sides, leaving the open ends on the left and the right for the drug to be administered (Morris, 2008). After the VALAP dried, the amoebae were observed under 10x magnification using a Nikon Eclipse E200 microscope with a Sony DFW-X700 camera.

The control and the experimental amoebae were both contained in a solution of pond water. Using the BTV imaging programming, still images of the control amoebae were taken for two minutes at intervals of fifteen seconds. The number of pseudopodia was then counted for each still image. The cumulative images were manipulated using Adobe Photoshop CS2 in order to put scale bars into the pictures. Nocodazole was dissolved in a solution of dimethyl sulfoxide, which enables the drug to quickly get into the system of the organism. The concentration of the nocodazole was 2 mg/mL. Gloves must be worn when handling the nocodazole to prevent any contact with skin if there is a spill (Morris, 2008).

In order to apply the nocodazole, the flow cell was placed on top of a paper toilet. A kimwipe was placed at one open end of the coverslip and 1/10 mL of nocodazole was administered at the other end. The kimwipe flowed the drug through the entire flow cell. This procedure was repeated three times so that the amoebae were immersed in the nocodazole. BTV was used again to capture still frames for two minutes at intervals of fifteen seconds. The number of pseudopodia for the amoebae was counted and recorded. The averages of the pseudopodia were found for the control and experimental amoebae. Adding together the number of pseudopodia for every interval and dividing by the total number found this calculation. The average number of pseudopodia for the control and experimental amoebae were plotted using a bar graph (Figure 2).

All slides were placed into the glass disposal container. The nocodazole was returned to the professor. Any kimwipes or paper towels that had been used were put into the trash. The microscope was shut off and covered.

## Results:

Pseudopodia are defined as regions of cytoplasm having two parallel opposite membranes in which independent cytoplasmic streaming occurs. The pseudopods extended from a cell body, which is a region of cytoplasm in which streaming was not connected to the bulk cytoplasmic flow. The cell body is viewed as a dark region that retained its shape. In Figure 1.1, the cell body is the circular end of the amoeba, acting as a base for the pseudopods. In Figure 1.2, the cell body of the amoeba is the dense region on the left with the pseudopods extending out to the right. Both the

control and experimental amoebae had cytoplasmic streaming, but the streaming appeared to have a faster rate in the control amoebae. The amoeba treated with nocodazole appeared to have stopped all movement for a brief moment until streaming began again. The control amoebae tended to have smaller pseudopodia, such as the ones extending downward, and one large pseudopod as in Figure 1.1. Contrariwise, the experimental amoebae had more long pseudopods than small ones (Figure 1.2). The control amoebae had larger pseudopods, averaging around seven pseudopods (Figure 1.1). Amoebae treated with nocodazole had long, thin pseudopodial extension (Figure 1.2). The average number of pseudopodia for the nocodazole-treated amoebae was nine. The difference in the average number of pseudopodia in the experimental versus control amoebae is two (Figure 2).

*Figure 1.1*

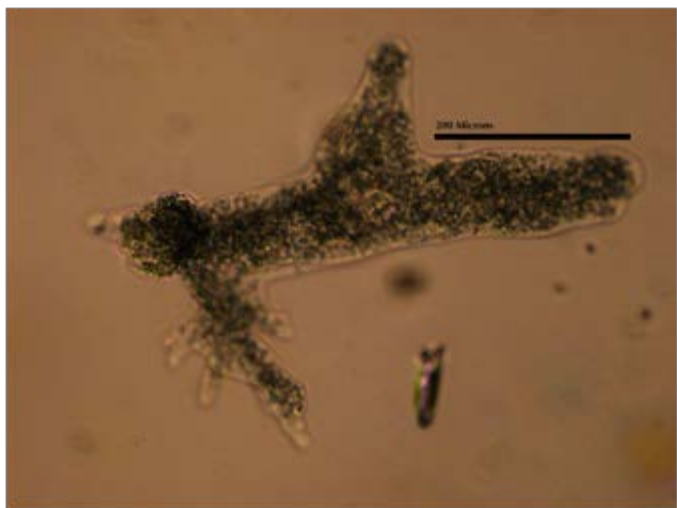


Figure 1.1: Observation of a control amoeba having seven pseudopods extending from a cell body. This picture was taken under 10x magnification on a Nikon Eclipse E200 on ICUC Leo (Scale bar = 200 microns).

*Figure 1.2*



Figure 1.2: Observation of amoeba after being treated with nocodazole, displaying nine pseudopods extending from a

cell body. This picture was taken under 10x magnification on a Nikon Eclipse E200 on ICUC Leo (Scale bar = 200 Microns).

Figure 2

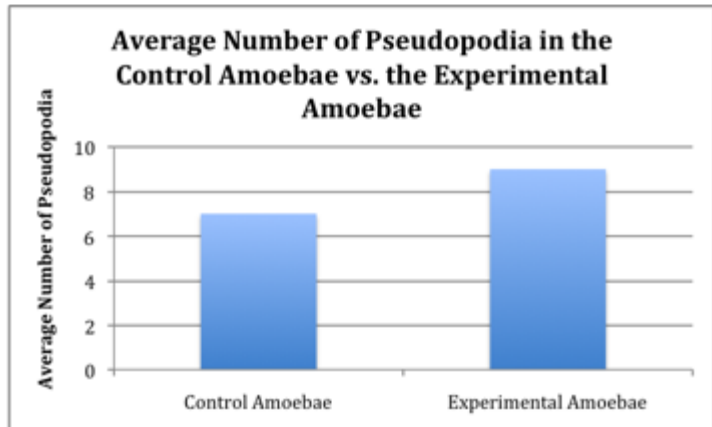


Figure 2: Comparison of the average number of pseudopodia in control amoebae and amoebae treated with nocodazole (n=9).

## Discussion:

The data from the experiment refuted that the amoebae would exhibit fewer pseudopodia after being treated with nocodazole. The average pseudopodial number was higher in the experimental amoebae than in the control amoebae. The difference in pseudopodia was only two, but the amoebae treated with nocodazole consistently have more pseudopodia. This reaction from the amoebae is due to the fact that the microtubules elongated after the treatment and were inhibited from retracting. The microtubules stayed in a paused state, varying only with slight intracellular movements (Vasquez, 1997). The control amoebae were able to form their microtubules to extend the pseudopodia, but also shorten the microtubules in order to extend the pseudopodia in a different direction. The cell regulates the microtubule-associated proteins, which in turn allows the cell to control the stability of the microtubules (Cooper, 2007). In the presence of nocodazole the cell cannot regulate these proteins, making the microtubules unstable. Microtubule instability can affect motor protein movement because nocodazole slows migration as well as disrupting focal adhesion disassembly (Schober, 2007).

Several errors could have occurred during the experiment. Maintaining the same amoebae after switching flow cells was difficult due to their amoeboid movement. The amoebae were never in the exact same place after being previously viewed. Since the amoebae were not being constantly observed, some cellular behavior may have been missed. If enough time had not passed since the drug was administered, the amoebae may not have showed the full effects of the nocodazole treatment. This error could have been the reason why the pseudopodial number in the

experimental amoebae was not that much higher than the pseudopodial number in the control amoebae. Additionally, more of the drug may have needed to be flowed through the slide in order to provide a concentrated solution. These errors as well as the results are the same for the collaborator because the data was equivalent due to the set of definitions.

If this experiment were repeated, more time should be taken to observe both the control amoebae and the experimental amoebae. By allowing more time, the error in the data will decrease because the average will be taken from a greater number. Observing the experiment for a longer time can also allow the drug to take its full effect on the amoebae. However, the control amoebae should also be placed in a solution of dimethyl sulfoxide so that the control amoebae are subjected to the same solution as the experimental ones. For future experiments, time could be a factor to see whether it affects the number of pseudopodia after the drug has been given. Using different concentrations of nocodazole could change the way the amoebae are affected. Other drugs could also be tested to compare how the pseudopodial number varies in comparison to the results with the nocodazole-treated amoebae.

## References:

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I collaborated with Dashawn Ealey in order to gather data for this experiment.

"I have abided by the Wheaton College Honor Code in this work."