

# Pseudopodial Lengths Decrease as a Result of Cytochalasin

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## Introduction:

As one observes a cell, a variety of dramatic behaviors can be noticed. These behaviors can be seen as just mere movements, and it doesn't take much to change these behaviors drastically. Cell behaviors can include cell movement, engulfing a particle, or even division. Actin, which is a major protein of the cytoskeleton in most cells, can play a large role in these different behaviors. These filaments are mostly found beneath the plasma membrane where they aide in mechanical support, a cell's shape, and cell movement. Actin filaments are able to grow by sub units or monomers, known as g-actin, assembling to either end of the polymer; although it is known that the polymer will assemble much quicker if the monomers are added to the barbed end. These polymers can also undergo depolymerization when necessary which can also have an effect on the behaviors exhibited by cells (Cooper and Hausman, 2007).

Different kinds of drugs such as cytochalasin or phalloidin can participate in changing a cell's behavior by interacting with the actin filaments. Specifically, cytochalasin is known for binding to actin and affecting its polymerization and elongation (Haidle and Myers, 2004). Cytochalasin will bind the barbed ends of the polymer which as mentioned is the quickest end for assembly, and this binding can result in changes in a cell's shape or the discontinuation of certain types of movement. This occurs because actin polymerization is required for these types of processes to be carried out (Cooper and Hausman, 2007). Movement can be an important factor to a cell, but can this drug really completely halt the process?

A common way for cells to provide their own transportation is through pseudopods. Pseudopods are projections that extend and contract by the reversible assembly of actin subunits into microfilaments. An organism which is known for its pseudopodial movement is amoebae proteus. This organism is large enough that it can be barely seen with the naked eye, but it has distinguishable features such as its pseudopods branching outward and its organelles which can be clearly seen with microscopic aide (www.wikipedia.com, 2008). This makes this specimen promising for observing affects of the drug cytochalasin on cell movement by observing the pseudopodial lengths.

In this study, it was hypothesized that after the addition of cytochalasin the pseudopodial lengths of the amoebae proteus would decrease due to the actin polymerization becoming blocked. The experiment consisted of amoebae which were exposed to the drug, and a group of amoebae which remained without exposure to the drug. Pseudopodial lengths were measured before and after the drug was administered, through this quantitative data and observations, conclusions were able to be made.

## **Materials and Methods:**

For this experiment to begin, two chip chambers needed to be assembled, one for the control of the experiment and one for the drug to be administered to. To assemble these chambers coverslip chips, created from breaking up coverslips, were placed in a square shape using forceps onto two clear slides. The organism of interest was the amoebae proteus; the amoebae were found using a dissection microscope and placed onto these two slides using a pasteur pipette. Coverslips were then placed on top of them to prevent the organism from drying out. If too much liquid was added to the slide, a kimwipe was used to remove the excess. Then to create a flow cell that enables the drug to be administered on one side of the coverslip, VALAP was used. Using a paintbrush, the VALAP was spread into a thick strip along the top and bottom of the coverslip.

To be able to observe these organisms in their chip chambers, the microscope Nikon Eclipse E200, which was used, needed to be aligned for Koehler Illumination. To complete this part of the procedure, the sample was focused on using the 10x objective lens; the field iris was closed so that its edges were able to be seen. The condenser of the microscope was focused so the field iris was focused on the sample plane; by using the positioning pins on the illuminator the field iris can be centered in the light path. At this point the field iris was reopened and the condenser aperture was adjusted until the contrast was appropriate.

Once aligned for Koehler Illumination, the computer Gemini was turned on along with its camera Spot 2. The

images of the amoebae from the control group and the experimental group were observed by taking three images each a minute apart using the Spot program. The lengths of the pseudopodia were measured at this point; pseudopodia were defined for this experiment as any protrusion from the main body of the organism which can be defined as the central region with similar width and height. The pseudopodia were measured by using the program Adobe Photoshop.

After the initial measurements were recorded, the drug cytochalasin was added to the experimental flow cell. The cytochalasin was added by filling a calibrated pipette 1/10 of a milliliter with the drug with a concentration of 40  $\mu\text{g/mL}$ , placing a kimwipe on one side of the flow cell, and adding a drop of the drug on the opposite side of the kimwipe. The last step was repeated two or three more times to ensure that the drug was added effectively. Fifteen minutes after the drug was administered, pictures were then taken as observations every minute for three frames, and the lengths of the pseudopodia were measured once again. Scale bars were created using the program ImageJ to show the size of the pseudopods in microns. This was completed by first measuring the size of the pseudopods in pixels and by using the conversion factor ten microns is equal to 8 pixels, the true length of pseudopods in microns were calculated and a scale bar was placed on them.

Pond water was added to the flow cell of the control. It was also added by filling a calibrated pipette 1/10 of a milliliter with the water, placing a kimwipe on the opposite side of the flow cell, and adding a drop of the water to the opposite side of the kimwipe. Fifteen minutes were also given for the sheer force of the pond water to take affect then observations were made, pictures were taken every minute for three frames, and the lengths of the pseudopodia were measured. The quantitative data that was collected for both the control and the experimental was averaged by adding each length of the pseudopods together and dividing by how many pseudopods were visible for all three frames before and after the drug was administered. This allowed for figures to be created, and it was used to compare the pseudopodial lengths of the amoebae of the experimental flow cell and of the control flow cell.

## Results:

There was an observed decrease in the pseudopodial length of the experimental amoebae proteus compared to the control amoebae before the drug was administered. The control amoebae had a much greater average pseudopodial length than the experimental group once the averages were calculated (figure 3). In figure 1 compared to figure 2, one can see that the pseudopods have decreased in size by a significant amount. The pseudopods of the experimental group do not stretch out as far as the control group; the pseudopods are also hardly noticeable in the experimental group

(figures 1 and 2).



Figure 1 – This image shows the amoeba of the control group before the drug cytochalasin was introduced into its environment. The scale bar indicates that its pseudopodial length for this particular pseudopod is 136.25 microns. There are two other visible pseudopods which are reaching out causing movement.



Figure 3 – This image shows the amoeba of the experimental group after the drug cytochalasin was introduced into its environment. There are only two visible pseudopods according to the definition. The pseudopods the organism has do not stretch out as far as the amoeba before the drug was added; they have decreased in size. The scale bar indicates that this particular pseudopod is 42.5 microns.

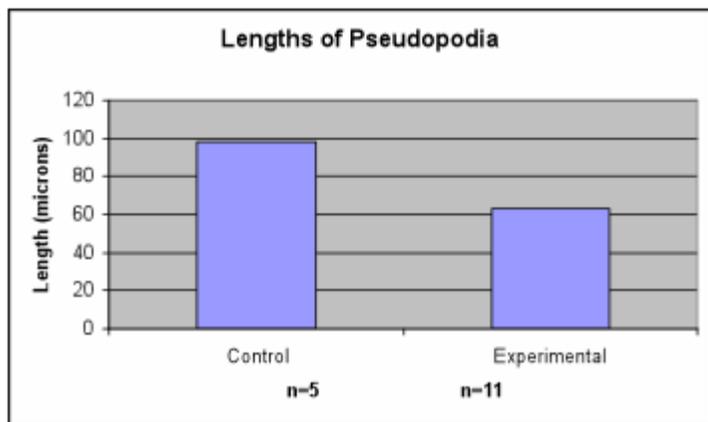


Figure 4 – The pseudopodial lengths of the amoebae were shown to have decreased after the addition of the cytochalasin. The experimental value was calculated by averaging the pseudopodial lengths from the three images which gave a total of n=11 pseudopods taken after the drug was administered. The control value was calculated by using the same method with the only difference being that there were only n=3 pseudopod lengths averaged.

## Discussion:

The pseudopodial lengths of the amoebae will decrease after the cytochalasin has been added. Based on the results that were collected during this study, this hypothesis can be supported. The observations that were made throughout the experiment along with the quantitative data that was collected show a dramatic decrease in the length of pseudopods which could hinder the cellular behavior of movement.

The quantitative data in figure 3 shows a statistically significant difference between the average pseudopodial lengths of the control compared to the average lengths of the experimental. This supports the idea that the polymerization of the actin was inhibited, and the actin could not elongate because of it (Haidle and Myers, 2004). The elongation of the actin would have caused the pseudopodial lengths of the drug-induced amoebae to continue to outstretch as it did before the drug was administered.

The lengths of the pseudopods were noticeably different in observations made during the experiment. Figures 1 and 2 show a comparison of two of the images; figure 1 is taken before the drug was administered, and its pseudopods were outstretching in each direction with great length. Figure 2 shows the amoebae after the drug was added to the environment, and it looks nothing like figure 1. The pseudopods are hardly visible, they have decreased in size as well as in width.

If repeated, the experiment should be refined. There were several sources of error that occurred while this experiment was completed. The control group only had the pseudopodial lengths of the first three images before the

pond water was added. This is due to the fact that once the pond water was added; the amoeba that had once been located had disappeared underneath the VALAP. There could be no measurements fifteen minutes after the pond water was added. Also to better this experiment the control group should have had Dimethyl Sulfoxide (DMSO) added to it instead of pond water for sheer force. This should be done because DMSO was the solvent that the drug cytochalasin was dissolved in; this could make for a more realistic control group.

For future experiments of this nature, one could consider taking images of the experimental group at certain times over a specific period of time before and after the drug was added. This would allow a rate to be calculated, and it would be possible to see if the drug has an effect on the pseudopods instantly or over a short period of time. Our experiment could not see this because there was a fifteen-minute wait between adding the drug and making observations; it was not possible to tell if the change happened slowly or extremely quickly.

The pseudopodial lengths of amoebae proteus can be decreased with the addition of cytochalasin. The quantitative data shows a difference which is large enough to be statistically significant, and the observations of the pseudopods decreasing and the amoebae moving slower after the drug was administered help to support this hypothesis.

## Works Cited

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I also collaborated with Mae Ciampa during this experiment.

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