Change in the Rate of Pseudopod Growth Under the Effects of Cytochalasin B

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Introduction:

Pseudopodia are temporary projections of the plasma membrane in eukaryotic cells (Cooper 2007). In order to define a domain as a pseudopod, it was decided that the plasma membrane domains of the pseudopodium must be parallel at some point. Amoebae have no internal or external skeleton, so in order to move, the amoeba must use pseudopodia. Amoeboid movement has existed in single celled organisms for billions of years, and it is a simple and straightforward concept. Amoebae use Cytoskeletal treadmilling and cytoplasmic streaming in order to crawl through their environment (Taylor 1978). The method they employ is called amoeboid movement. Cytoplasmic streaming and amoeboid movement are fundamental cellular functions where cytosol and its constituent particles stream in and out of pseudopodia (Taylor 1978). The main cytoskeletal subunits, the actin cortex and the microtubules, polymerize at one end and de-polymerize at the other. Cytoskeletal treadmilling allows for constant motion in the actin cortex and the microtubules (Cooper 2007). The actin cortex polymerizes G actin on the pointed end of the filaments, and it depolymerizes on the barbed end of the actin filament. The fact that the actin cortex operates on the basic concept of treadmilling is important because if one of the main cytoskeletal subunits can exhibit treadmilling, then this allows the cell to exhibit thixotropic properties, which means that the cell exhibits behaviors of both liquids and solids.

Actin filaments near the plasma membrane can also interact with myosin. Myosin is a motor protein that interacts with the actin cortex to cause contractions. When the actin cortex interacts with myosin in an amoeba, it causes the pseudopod to contract. (Taylor 1978).

The behavior of amoebae under the effects of cytochalasin B has also been studied in detail. For this particular procedure, cytochalasin B is a drug that is dissolved in a solution of dimethyl sulfoxide, or DMSO. DMSO is a powerful solvent, so it allows the drug to be delivered directly inside the plasma membrane and into the cytosol, thus its effects can be observed immediately. Cytochalasin B inhibits the cytoplasmic streaming of amoebae by depolymerizing the microfilaments of the actin cortex and causing the cell to lose some of its internal structure (Bradley 1973). This makes it more difficult for the cell to carry out its normal functions, and this shows that the treadmilling of the actin

cortex is extremely important for pseudopodial movement (Bradley 1973). If the actin cortex cannot treadmill normally, then the cell loses its ability to function properly (Bradley 1973).

The hypothesis tested for this experiment was: If 150 microliters of cytochalasin B are added to a flow cell containing an *amoeba proteus*, then that amoeba specimen will expand its pseudopodia more slowly over time. This hypothesis is significant because it examines the important cellular behavior or pseudopod expansion and retraction. Without normal levels of actin filament treadmilling and pseudopod growth, the amoeba would not be able to expand and contract its pseudopodia, and therefore it would not live for very long. Also, *Amoebae Proteus* are very easy to study. They are easy to image, easy to maintain, and they contain a high number of cytoplasmic particles, which make them an ideal subject for study in this experiment. In this study, the amoebae were observed in the presence of Cytochalasin B, and their pseudopodial movements were measured using flow cells, microscopy, and Photoshop and Image J software.

Methods and Materials:

There were a number of important pieces of equipment used in this experiment. 2 slides with cover slips and cover slip chips were used to construct a flow cell. The cover slip chips are placed on the slide, and the cover slip is placed over the chips. Then the chamber is sealed with heated VALAP applied with a paintbrush. Once the specimen is prepared, a Nikon Eclipse E200 microscope is used to image the sample. 300 microiters of 40 microgram per ml cytochalasin B are added to the flow cell in order to observe the changes in cell behavior predicted in this experiment. 2 *Amoeba Proteus* are used to test the effects of cytochalasin B. a pipette is used to add 50 microliters of cytochalasin B at a time. An IMAC computer is used to run the BTV, Image J, and Adobe Photo Shop CS software. Finally, a Sony DFW-X700 camera was used to capture frames of the amoeba specimens. These supplies were supplied by the professor and by the biology department at Wheaton College.

First, the slides were prepared so that the amoeba could be viewed and fluids could be passed through the slide at the same time so that the drug used for this experiment could be tested. This setup is called a flow cell. First, cover slip chips were placed in a ring on the top of the slide. There should be 4-6 of these chips on each slide. Next, the solution containing the amoeba was pipetted onto the slide by the professor. Next, the cover slip was quickly placed on top of the slide and cover slip chips so that no air bubbles formed. Next, the pre-heated VALAP was painted onto the two edges of the cover slip that were flush with the narrow part of the slide. The VALAP was painted onto only those two

edges in order to allow fluids to flow in and out of the cell created by the cover slip chips, the slide, and the cover slip. Once the flow cell was made, these steps were repeated for a second slide. Then, the flow cells with the amoebas were viewed under the microscope. It was then possible to observe and measure pseudopodial movement in the amoeba. When the cytochalasin was applied, 50 microliters of the 40 micrograms per ml of cytochalasin solution were applied to the side of the flow cell. A kimwipe was placed on the opposite side of the flow cell in order to diffuse the cytochalasin throughout the flow cell as quickly as possible. Gloves were worn at all times in order to insure that the cytochalasin B did not penetrate the skin. The application of Cytochalasin B is repeated two more times for each slide to ensure saturation.

In order to measure the rate of psuedopodial expansion, a domain of the pseudopod was chosen, and a time-lapse exposure was taken over a period of thirty seconds. The way each pseudopod was chosen was by using the most linear growth pattern possible in order to measure the length of each cytoplasmic projection using a straight line. This selection method was especially useful in samples with multiple pseudopodia. One frame was taken every five seconds in BTV. Three time-lapse videos were captured for each slide. The first were the control exposures and the last two for each slide were experimental exposures.

The rate of expansion was measured by dividing the length of pseudopod expansion by the length of time. The length was determined by using a scale bar in the Image J software program. Cytochalasin B was then added to both flow cells, and this was repeated three times for both slides so that there would be 150 microliters of cytochalasin B on each flow cell, which is the maximum capacity of each flow cell. This was also done to ensure that the samples would be subjected to the maximum effects of the cytochalasin B.

The imaging began at 3:55 PM and the control images were taken. Cytochalasin B was applied to slide A at 4:05 PM. Three images of slide A were taken at 4:10 PM. Next, 50 microliters of cytochalasin B was applied to slide B at 4:24 PM. New images were taken for slide A at 4:37 PM and for slide B at 5:00 PM. This allowed for a control image for each slide, a five-minute cytochalasin B image for each slide, and a thirty-minute cytochalasin B image for each slide.

Once all the images were taken, they were re-opened in Adobe Photoshop in order to accurately measure pseudopod growth. Each time-lapse exposure was manipulated so that the first image was darkened and placed on top of the final image in two layers. Then, the opacity of the last image was reduced so that the top image could be superimposed on top of the more transparent bottom image. Then the two layers were flattened so that the length of pseudopod growth could be measured. The measurement was recorded in pixels using a scale bar in Image J. Then, the distance in pixels was converted to micrometers by using the stage micrometer. The distance of expansion was divided

by thirty seconds in order to obtain a measurable rate (micrometers per second).

Results:

The results of the cytochalasin B testing on amoebae showed that cytochalasin B has a significant impact on the pseudopodial motility of amoebae. The mobility of the pseudopodia decreased after five minutes, and it decreased even more after thirty minutes. The rate of decrease in mobility was very steady all the way from the control group to the thirty-minute exposure group.

The T- test used to determine the statistical significance of the data was a two sample assuming unequal variances T-test. The pseudopod formation rate after 5 minutes (M=2.516, SD=0.787) did not differ significantly from the control pseudopod formation rate (M=3.178, SD= 0.825), $t(10)=2.23$, p=0.18 However, pseudopod expansion rate 30 minutes after cytochalasin B was administered (M=1.783, SD=1.01) did differ significantly from the 5 minute samples t (10)=2.23, $p=0.25$. The rate of pseudopod expansion 30 minutes after the drug was administered was not significantly different from the 5 minute group, $t(10)=2.26$, p=0.194The results of the control group differed from the 5-minute specimens by 20.8%. The difference between the results of the 5-minute group and the 30-minute group differed by 29.1%. Essentially, the rate of change between the three groups was quite linear, and the drug activates gradually over time, not exponentially.

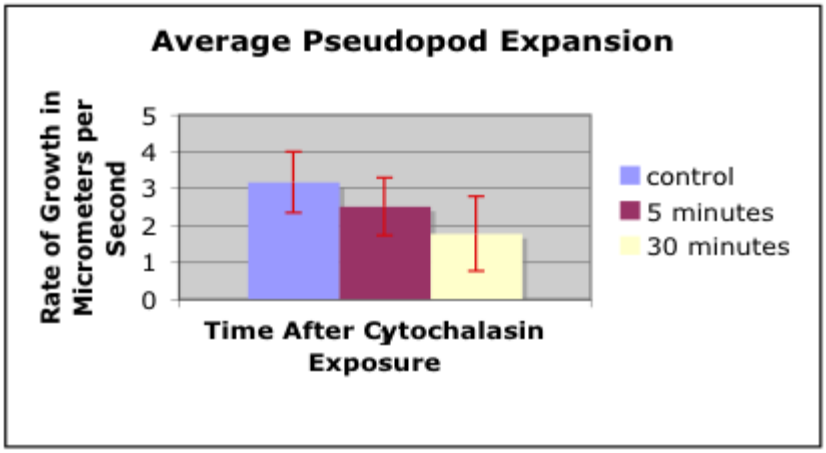


Figure 1: Average pseudopod expansion in micrometers per second

This figure represents the average expansion of the pseudopodia for each group in micrometers per second. The blue portion represents the control amoeba sample, the purple portion represents the 5-minute exposure to cytochalasin B, and the yellow portion represents the 30-minute exposure to cytochalasin B. The red error bars represent the standard deviation for each exposure. N=6 for all trials

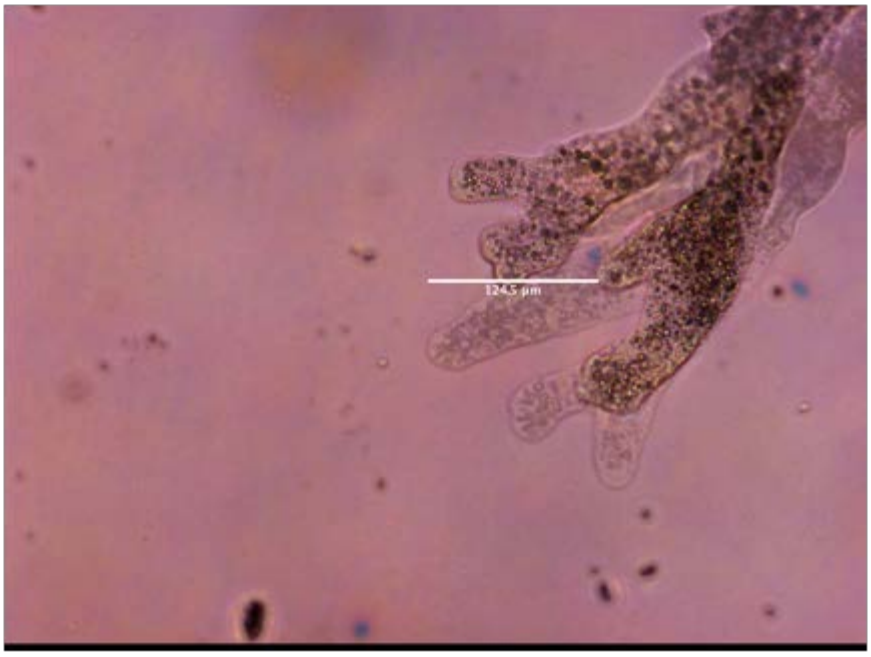
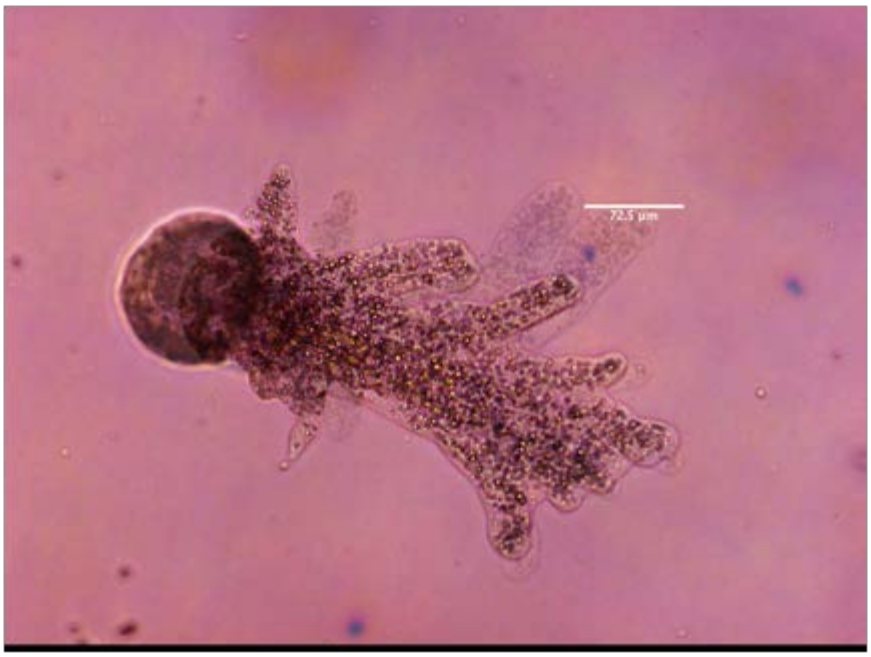


Figure 2: superimposed Image of Control Amoeba

Here, a healthy pseudopod can be seen growing throughout the time-lapse exposure, and the pseudopodial growth can be measured with a straight line, giving the observer reliable quantitative data regarding the length of the pseudopodia.



. Figure 3: superimposed image of Amoeba after treatment with cytochalasin B

Although the pseudopod growth in this image appears to be mostly normal, there is a domain on the left side of the image where no growth can be measured. A large bubble-like structure can be observed here.

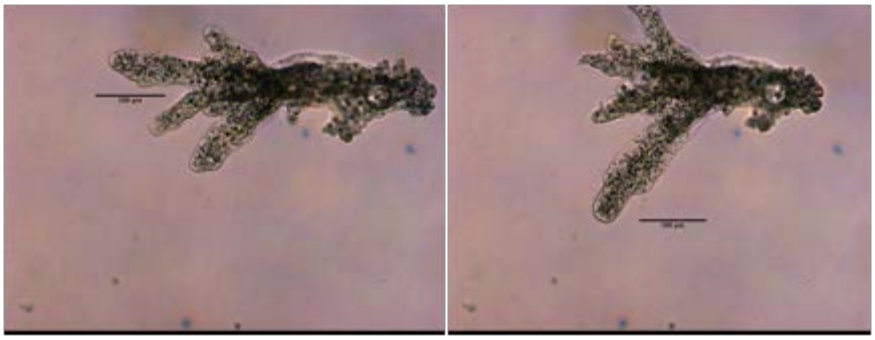


Figure 4: demonstration of normal pseudopodial movement.

The specimen is able to extend its cytoplasm and plasma membrane outwards in a classic amoeboid motion, it is able to stream its cytoplasm into the membrane projection it creates, and it has covered a notable amount of distance during the 30-second time lapse.

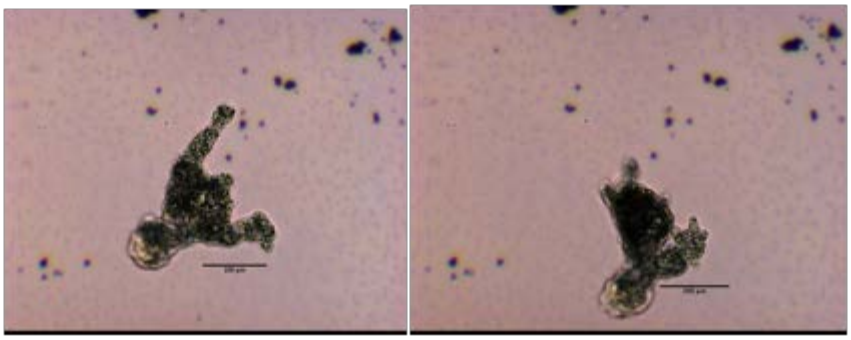


Figure 5: demonstration of pseudopodial movement after the application of cytochalasin B.

Here, the specimen is unable to undergo normal amoeboid movement, the plasma membrane does not noticeably expand or contract, and no notable cytoplasmic streaming occurs either. There is some movement, but this is due to the fact that the amoeba is floating in the fluid in the flow cell. Any movement it undergoes is dependent on the flow of liquid inside the flow cell, and it does not appear to be capable of independent motion.

Discussion:

The hypothesis for this experiment was supported by the results of the cytochalasin B testing. The average movement of the pseudopodia in amoebas not treated with cytochalasin B was slightly higher than the amoebas measured five minutes after being treated with cytochalasin B. However, the pseudopodial movement in the samples that had been imaged 30 minutes after the application of cytochalasin B was significantly lower than the control samples. This shows that the amoebas moved noticeably slower over time when cytochalasin B was applied. It is most likely that this decrease in speed is a direct result of the de-polymerization of the actin cortexes of the amoebae as a

result of exposure to cytochalasin B.

Also, there were other results aside from the strictly quantitative data regarding the growth of the pseudopodia of the amoebae. In one of the specimens treated with cytochalasin B, a bubble composing a large percentage of the cytosol had formed inside the plasma membrane. The amoeba was undergoing pseudopodial movement, but it was dragging the bubble around behind it, and it was clear that this bubble hindered the movement of this particular amoeba. The most likely cause of formation of this bubble was the detachment of the actin cortex from its anchoring proteins and the plasma membrane. The proteins that bind the actin cortexes and adherens junctions are called alpha catenins and cadherens. (Cooper 2007). Alpha catenins bind to the intracellular domains of the cadherens, and the catenins hold the actin filaments in place. (Cooper 2007). The most likely explanation for this intracellular bubble is that the cytochalasin B de-polymerized the alpha catenin/ cadhheren binding complexes before it de-polymerized the actin cortex of that particular amoeba, causing the actin filaments to cluster together in the cytoplasm and form a sphere.

There were definitely some possible sources of error in this experiment. First of all, there was no true control. A true control would have been injected with only pond water and DMSO, but due to the difficulty of catching the amoebae, there was not enough time or resources to institute a proper control in this experiment. Second, the concentration of cytochalasin B could have been higher or lower than 40 micrograms per ml, which would cause the average rate of pseudopod growth to be inaccurate. Also, it is possible that the five-second time lapses were lesser or greater than five seconds, causing the amount of measured pseudopod growth to either be too low or too high. In addition, it is possible that the decrease in the growth rate was not actually a result of exposure to cytochalasin B, but the result of the amoebae sitting in the flow cells with insufficient food and oxygen for too long. If this were the case, then all the data collected in these trials would be nullified. Despite all of these potential blunders, great lengths were taken to ensure that none of these sources of error occurred.

There are a number of future possibilities for this test as well. If it were done again in the future, it would be extremely helpful to have an increase in sample size. Because only two slides with amoebas were imaged in this experiment, the hypothesis would have been supported more strongly with several times as many specimens as possible. The reason that the sample sizes were so small was because it was extremely difficult to pipette the amoebas onto each slide. If there had been more time to pipette amoebas, then more samples could have been imaged. Also, It would have been nice to not have to treat all the amoeba samples with cytochalasin B, but again, it was difficult to pipette each amoeba onto each slide so the amoeba could be easily viewed.

Also, there should absolutely be future experiments done regarding the subject of the effects of cytochalasin B on amoeboid movement. Any future experiments could be done regarding the effects of other drugs such as nocodazole

on the behavior of the polymerization and de-polymerization of the actin cortex or other cytoskeletal structures such as the microtubules. In addition, further experimentation could be done on different cell behaviors that are dependent on the actin cortex, such as cytoplasmic streaming and lamellipod growth rates. Also, different measurements could be taken, such as the number of pseudopodia on an amoeba with cytochalasin B applied to it. The possibilities are very promising, and perhaps some of the data and trends could even be applied to cells in organisms other than amoebae.

Cytochalasin B is a powerful drug that noticeably changes cell behavior. It serves as an excellent tool to observe the role of the actin cortex in a cell, and it is also a powerful reminder that if one small subunit is altered or removed inside the cell, there is often an organismal response to that stimuli. When one component of any system is thrown out of balance, the entire system changes, and it can become in danger of failing. The cell is a perfect example of this phenomenon, and it is helpful to keep this in mind when dealing with cellular functions.

Bibliography:

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4. Rob Manguso was my lab partner for this assignment. Our graphs and statistical data are the same due to the fact that we recorded the same data.