

Pseudopodia Average Number Decrease in Treatment with Nocodazole in Amoeboid *Proteus*

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Introduction

Amoeboid Proteus is a unicellular organism, which can lengthen its protruding cytoplasmic extensions to reach over one millimeter when maximally extended (Cooper & Hausman, 2006). These extensions, called pseudopodia, provide a method of motility for the eukaryote. This sort of movement of the *Amoeboid Proteus*, is one that occurs from a transition of actin cytoskeletal filaments from gel to sol and is known as amoeboid movement (Class Notes). Pseudopodia are able to form into these extensions by the reversible assembly of actin subunits into microfilaments. Pseudopodial extensions continue to lengthen until the actin filament assembles into an actin network. By this reassembly *Amoeboid Proteus* is able to develop a motile function, an ability found in some animal cells such as white blood cells (Cooper & Hausman, 2006).

These pseudopodial extensions form in response to environmental stimuli and are short-lived as they continue to reassemble by polymerization and depolymerization mechanisms (Cooper & Hausman, 2006). These structures extend from the leading edge of the moving amoeboid in order to provide the motility the cell needs (Ueda & Ogihara, 1994). The pseudopodia are present due to cross-linking actions of actin filaments into an actin network (Cooper & Hausman, 2006). For the purpose of this experimental investigation, pseudopodia were identified as long thin extensions of the cytoskeleton with a curved terminal end protruding away from the main cellular body in a manner that would make it easily identifiable. The crawling movement of the eukaryotic cell depends on this process of actin filament assembly that expands the cytoskeleton gel at the cells leading edge (1-5) (Miao, 2008). By this action, the actin filaments of the cytoskeleton provide a mechanism of tension, while the microtubules act as a means for compression in the structural

cytoskeleton of the cell.

Microtubules are a vital component of the cytoskeleton, along with actin and intermediary filaments, which contribute to the structural support of the cell. Microtubules aid in the cellular processes of cell division and vesicular transport (Ueda & Ogihara, 1994). They are polymers composed of α and β tubulin dimers which are able to polymerize end to end, with the α tubulin of one dimer coming into contact with the β tubulin of another dimer. These tubulin subunits construct a long rigid tube-like structure which remains hollow on the inside while extending from its origin, the centrosome, to the periphery of the cell (Cooper & Hausman, 2006). The β subunit is exposed during this process of polymerization and becomes bound to a GTP molecule. The GTP bound to β -tubulin is bound to be converted to GDP not long after assembly, with the most prominent difference between the two being the fact that the structure GDP-tubulin makes it more prone to depolymerization. Tubulin will only add onto the end of the microtubule in the GTP- bound state, the cap protects the microtubule from disassembly. Depolymerization, known as catastrophe, occurs when the leading end of the microtubule undergoes hydrolysis and converts to GDP. There is a constant switch from growth to catastrophe going on in the microtubule (Cooper & Hausman, 2006). In order for a pseudopodial extension to occur an interplay between actin and microtubule filament polymerization and depolymerization must occur. Actin depolymerizes followed by microtubule polymerization resulting in an outward extension of the plasma membrane, the actin then repolymerizes or reassembles as a means of structural support of the pseudopodium which then proceeds to extend.

These microtubule dynamics which result in the polymerization and depolymerization of this cytoskeletal element can be affected by drugs. Nocodazole, is a microtubule polymerization inhibitor, which is know to block the polyermization of tubulin and is therefore known as a microtubule depolymerizer. Nocodazole is often used for cancer treatment since it intervenes with polyermization and depolymerization of cytoskeletal filaments and therefore hinders cell division (Vasquez et al. 1997). Aware of this background information, we hypothesized that when the drug nocodazole was added to the experimental slide, the number of pseudopodia of the *Amoeboid Proteus* would decrease over time while the control slide, used as a means for comparison, would not significantly change in number of pseudopodia over time.

Methods and Materials

First we obtained two slides and set up the traditional flow cell environment by placing three to four glass chips on both of the slides. We then obtained a few *Amoeba protei* and placed them on each of the slides. We then covered the organisms in the pond water environment with a coverslip and used VALAP to seal the flow cell on two sides: the upper and lower sides, while leaving the left and right sides open.

We then located the amoeboid of interest for experimentation on each of the slides (labeled “1” for control and “2” for experimental) and placed a small black dot on the coverslip with a black sharpie marker near the location of the amoeboid of interest as a reference point for later observation. Since more than one organism was present on each slide, our result would be thrown off if we first followed the development of amoeboid 1 and then mistakenly observed and recorded the development of another, for example amoeboid 2, or 3.

Next, we placed the control slide on the microscopic stage of a light microscope, Nikon Elipse E200, and focused on our organism of interest at a magnification of 4X on the BTVimage program at the Virgo Station of the ICUC at Wheaton College. Normally, an ocular magnification of 10X has been preferable since more detail is shown, however at this magnification we could not view the entire amoeboid, therefore hindering us from being able to count the number changes in pseudopodial extensions. We took pictures of the change in pseudopod number of the control slide at an interval of 1 minute for 21 minutes and recorded those numbers. The type of camera used to capture these images was a Sony Digital interface. These recorded numbers were then used to determine the average number of pseudopodia displayed by the *Amoeboid Proteus* at normal, pond water conditions and served as a mean of comparison with the experimental group/

We then obtained Nocodazole at a 2 µg/mL concentration in a test tube using a pipette. We flushed the drug through the flow cell environment using the pipette to localize the drug at the left side of the coverslip, and then using a kimwipe at the right edge of the coverslip to soak up the remaining solution. We did this three times to ensure that the fluid drug was fully transferred into the flow cell environment. By this fluid transfer procedure, we aimed to replace the 150 µL of pond water surrounding the organism with the 2 µg/mL concentration solution of the drug. The Nikon Elipse E200 microscope was set for a Koehler illumination to obtain the most detailed images possible.

Using the same program, BTVimage, we viewed the experimental slide and captured frames every minute for fifty-eight minutes. We continually recorded the number of pseudopods present at each time interval and graphed the results using the Microsoft Excel 2006 program. We then used imageJ and Adobephotoshop to compile the photograph images of the control and experimental slides at the first minute of observation as well as the last. For the control this was at minute one and twenty-one, while for the experimental group it was at minute one and fifty-eight. The data was then analyze and graphic displays were constructed as means for comparison between the number of pseudopodia present over time in the control (slide 1) and those exposed to the treatment of 2 µg/mL solution of Nocodazole (slide 2).

Results:

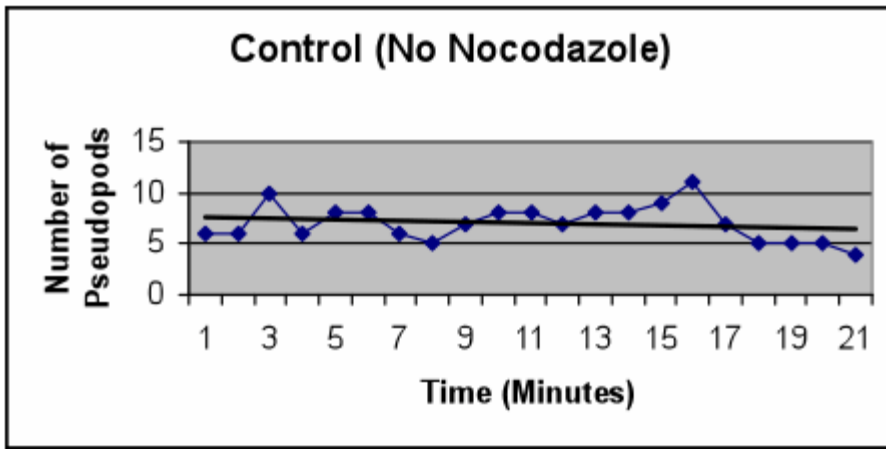


Figure 1. Change in pseudopodial number over a period of twenty-one minutes taken at intervals of one minute. A trendline is used to demonstrate the consistency in the results of the control slide of *Amoeboid Proteus*. (n=1)

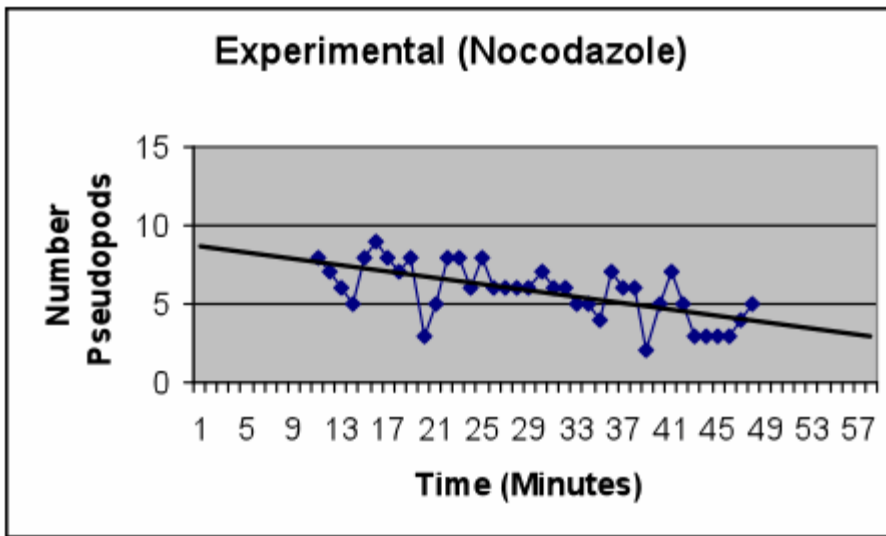


Figure 2. Change in number of pseudopods demonstrated in *Amoeboid Proteus* on the experimental slide treated with the 2 μ m/ml solution of Nocodazole and recorded at intervals of one minute for fifty-eight total minutes, line of best fit included. (n=1)

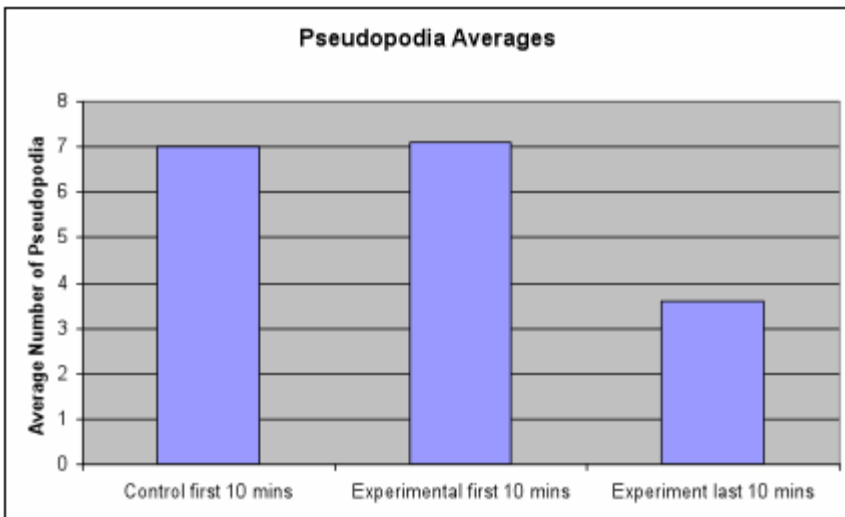


Figure 3. Comparison of the average number of pseudopodial extensions in *Amoeboid Proteus* during the first ten

minutes of the control slide, the first and last ten minutes of the experimental slide treated with 2 μ m/ml concentration of Nocodazole.



Image 1. The pseudopods displayed on slide 1 (control) at 2:56 pm at a 4x magnification



Image 2. The pseudopods displayed on slide 1 (control) at 3:16 pm at a 4x magnification

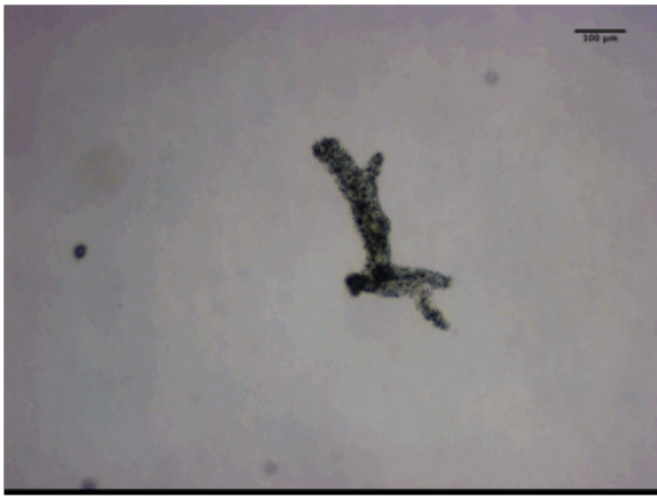


Image 3. Pseudopods displayed on slide 2 (experimental) at 3:21pm at a 4x magnification



Image 4. Pseudopods displayed on slide 2 (experimental) at 4:19 pm at a 4x magnification

After recording the experimental slide for fifty-eight minutes, to get the necessary amount of observational change in pseudopodial numbers and the control slide for twenty- one minutes, we used line graph visual displays as means of comparison between the control and experimental groups. The linear trend line added to the line graph for the control group (pond water environment and no Nocodazole) shows a very consistent trend from minute one to minute twenty-one as displayed in *Figure 1*. The control *Amoeboid Proteus* exhibited a range in pseudopodia number from four to eleven extensions, an interval consistent throughout twenty-one minutes of observation.

The experimental results, as shown in the line graph appear similar to those obtained from the control slide, however the trend line visible in *Figure 2* displays a negative correlation from minute one of pseudopodia number recording to minute fifty-eight of recording. This means that the slope shows a decrease in pseudopodia number over

the fifty-eight minute time span. However, the slope of the trend line is not very steep. To make the data findings more visibly evident the use of *Figure 3* is pertinent to a more comprehensive understanding.

An average of the pseudopodia numbers in the first ten minutes of observation was taken for the control, pond water only slide which came out to seven, while the average of pseudopodia number for the first ten minutes of the experimental Nocodazole slide was very similar at 7.1 pseudopodia. The median was also seven and the mode eight for the control group, while the median was six and mode eight for the experimental group. The control group ranged from displaying between four and eleven pseudopods at one time, while the experimental ranged from two to nine. The two averages (means) displayed as bars in *Figure 3* are significantly larger than the average pseudopodia number in the last ten minutes recordings of the experimental Nocodazole slide at 3.6 pseudopods.

Images 1-4 were used as a means to describe a qualitative difference in the pseudopodia appearance between the control and experimental groups. In the control, the amoeba consistently displayed long and thin extensions throughout the twenty-one minutes of observation as seen in *Image 1* and *2*, while the experimental group the pseudopodia initially displayed shorter, more pointed extension at the beginning stages of drug exposure as in *Image 3* but transitioned to being much shorter and had a great width towards the last ten minutes of observation (from around the 49th minute of Nocodazole introduction on) as displayed in *Image 4*. These shape changes however occurred gradually from the beginning of the Nocodazole solution and altered more over time.

Discussion

These results support our hypothesis that when the drug Nocodazole was added to the experimental slide, the number of pseudopodia on the *Amoeboid Proteus* decreased over time while the control slide, used as a means for comparison, did not significantly change in number of pseudopodia over time. Previous studies support our findings as well by concluding that the microtubules are a vital part of the cytoskeleton and without them actin reassembly is affected and delocalized from the periphery of the cell (Mikhailov & Gundersen, 1998). The addition of Nocodazole in this specific study also supported our hypothesis by demonstrating that the drug treatments resulted in significant reduction in growth and shortening rates of the pseudopodia while producing less dramatic effects on the growth and catastrophe components of dynamic instability in the microtubules (Mikhailov & Gundersen, 1998). Yet again, our results are supported and explained as an overall decrease in microtubule turnover after Nocodazole treatment which indicates a mitotic block is occurring due to a reduction in microtubule dynamic turnover (Vasquez et al., 1997).

With the compression component of the cytoskeleton inhibited, the pseudopodial formation was lacking the structural help of microtubules resulting in a decrease in number formed. This numerical decrease in pseudopodial

extension can cause motility problems for the cell since this is a pertinent characteristic for the walking ability of *Amoeboid Proteus*. Previous research demonstrates that pseudopodia on amoeboid are necessary motility components for the cell and that the amoeba organism utilizes the microtubule filament mechanisms of polymerization and depolymerization for structure and walking (Cameron et al., 2007).

Although not evident from our numerical data, the structure of the pseudopodia extension changed from being longer, finger-like extensions to shorter, much more curved, bump-like extensions. Although the line graphs resemble one another, and at first it is difficult to analyze the results and notice a difference in the trend lines the bar graph works to show the evident results in pseudopodial decrease with the addition of Nocodazole. The negative correlation of the slope in *Figure 2* shows a general decrease in pseudopodia number over the fifty-eight minute time period. To make the results more clear, and show that they strongly support our hypothesis, *Figure 3* was constructed which clearly shows that the average pseudopodia number of the first ten minutes of the *Amoeboid Proteus* treated only with pond water is very similar to the average number of pseudopodia in the first ten minutes of experimental treatment of the *Amoeboid Proteus* treated with two micrograms per milliliter of Nocodazole. The most drastic change could be seen in comparison of the first ten minutes of the experimental and control groups with the last ten minutes of the experimental since at this point the drug treatment had taken full effect. As stated earlier, although the numbers do show a decrease in pseudopodia number, a shape conformational change was observed that could not be recorded quantitatively. This could be seen from the captured images shown in the images captured.

The experimental error must always be taken into consideration in observations and tests like these. The experimenter observing, counting, and recording the pseudopodial number change must remain consistent in this procedure, however, this was a subjective method using the definition of a pseudopod (a surface extension of the cytoskeletal filaments used for cell motility). The identification and categorization of pseudopodia was also a difficult factor in the experiment. With a definition established prior to beginning of observation, the identification could not remain objective throughout the experiment since the size and shape of the cellular protrusions constantly changed, challenging our prior definition. Also, the sample size of our experiment was very small (n=1) for both the control as well as the experimental group therefore if either of these subjects were irregular in terms of shape, cytoskeleton elements, pseudopodia formation, or pseudopodial motility, the results of our experiment would be thrown off.

In order to observe the conformational changes and size differences due to cytoskeletal effect due to the addition of a microtubule depolymerizing agent further investigation is necessary. A further study on the subject would be to measure the surface area changes in pseudopodia with the addition of the drug treatments. By outlining the area of one pseudopod and finding the volume and the volume change from one pseudopodium in the first ten minutes of the drug

addition in comparison to the last ten minutes. This is a valuable proposal for future experimentation on the topic.

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