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The Effect of Cytochalasin B on the Movement of Cells: Pseudopodial Movement in Amoeba Proteus

Nkululeko Phakela

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I. Introduction

Cells are able to move from one place to another. This is more visible in unicellular organism since the movement is more drastic as opposed to multicellular organisms where the movement would only be intracellular most of the time,and not from one position to another. In this experiment I studied the movement of cells from position to position. Cell movement in either unicellular or multicellular organisms is made possible by the cytoskeleton. The cytoskeleton provides structural framework for cells, determines cell shape, organization of the cytoplasm and it is responsible for the cellular movement observed as explained by Geoffrey and Robert (2004). "The Cytoskeleton appears as a maze-like network of hollow fibers, extending the organelles in a kind of three-dimentional web."(Simmons) The movement can either be of organelles within the cell from one place to another,or it can be of the cell from one place to another as in the case of unicellular organisms like amoeba. The intracellular movement that is visible within cells includes, "vesicle movement between organelles and the cell surface, cytoplasmic streaming, movement of pigment vesicles for protective coloration, discharge of vesicle content for water regulation in protozoa, cell division which includes cytokinesis and movement of chromosomes during mitosis and meiosis" (Biology Project in Arizona University).
The cytoskeleton is made up of three principal types of filaments that cause this movement. These are actin Filaments, intermediate filaments and microtubules. In this experiment I studied the effect that actin filaments have on the movemvent of the cell. The drug Cytochalasin B depolymerizes actin and thus is expected to slow down cellular movements when added to the amoeba. In this experiment I studied the movement in the genus species *Amoeba Proteus.* "These relatively large protozoans use mobile extensions of the cytoplasm called pseudopodia for movement and food capture." The fact that *Amoeba Protei* use pseudopodia for movement helped to quantify my experiment that looked at the movement of amoeba relative to the movement of these pseudopodia in the *Amoeba Proteus* cells that I was studying.
I looked at different time lapse movies of the amoeba on a slide, and observed whether the amoeba moved by observing the distance that the amoeba had moved from its original position.Another way I defined movement was to look at the difference in the length of a pseudopod, at differerent positions, and different times. I defined a pseudopod as an extension of the cell membrane that has a length to width ratio of 1.5:1 or the part of the ratio that is the length could be more than1.5.
My hypothesis is thus the drug Cytochalasin B slows down cellular movement when added to the *Amoeba Protei* by depolymerizing actin in the cytoskeleton of *Amoeba Protei*.

II. Materials and Methods

MATERIALS

Cells Used, Software and Equipment

- Amoeba Proteus
- Nikon Eclipse E200 light Microscope
- Spot Insight QE camera
- Nikon SMZ660
- Spot 3.5.5 software
- DAGE MTI-DC200

METHODS

Control

I prepared a slide that has a chip chamber. I then looked at *Amoeba Protei* under a Nikon SMZ660 light microscope and observed the live images of magnified amoeba on a time lapse MTI-DC200 software, so that I could get a bigger picture of where the amoeba was. I then located the amoeba that I wanted to pull out of the culture dish of amoeba, and used a pastuer pipette to pull out amoeba. I pressed the knob at the top of the pipette, put the pipette in the culture jar close to the surface of the water, and pulled some liquid from the pipette. Then I squeezde the pipette a little and directed it towards the amoeba that I wanted to pull out of the culture. I pulled the amoeba out once I had the mouth of the pipette on top of the amoeba .I then put this on the slide and covered it with a cover slip, adding enogh water under the cover slip to cover all of it. Afterwards I looked at the amoeba adjusted to Kohler illumination under a light microscope at a magnification of 400, using a 40X objective lens.

I took sequential images of amoeba moving. From these sequential images I extracted the first and last image which had a difference in time of 5 minutes. Then i pointed out one pseudopod and measured its length and width using a software program called ImageJ. Using ImageJ I was able to measure from one part of the pseudopod, a pseudopod being an extension of the plasma membrane that has a length to width ratio of 1:1.5 or less. to another and then store the results that I had. I then looked at the sequential images and looked at where this pseudopod ended up and measured its lengthand width after 5 minutes, andthis was the tenth picture.

Experiment

I did the same procedure as above while I was getting amoeba from the culture and preparing the chip chamber on the slide. Once I had the amoeba in the pipette, I added a drop of 1.0ug/ml cytochalasin B and a drop of water,and this reduced the concentration of cytochalasin B to 0.5ug/ml since it was diluted by the water. I then put on the cover slip and observed the amoeba under the Nikon Eclipse E200 light microscope.

I took sequential images of amoeba movement after I added the drug, cytochalasinB at a concentration of 0.5ug/ml. From these sequential images I extracted the first and last image which had a difference in time of 5 minutes. Then i pointed out one pseudopod and measured its length and width. I then looked at the sequential images and looked at where this pseudopod ended up and measured its length and width after 5 minutes. The results are seen below.

Control

The 5 minute sequential images showed that the amoeba proteus was moving since it has traslocated from one place to another. The pseudopods had also changed in shape and size and this was quantitated by the measurement of their lengths and widths. It is visible from picture 1 and picture 2 that the amoeba moved from one place to another, and thus there has been movement, which iscaused by part of the cytoskeleton, Actin filaments.

After adding the drug, the amoeba was moving faster than before adding thedrug within the same amount of time. The amoebae moved from within the focusof the camera faster after adding the drug than before adding the drug, wherethe amoebae would take longer to move out of focus of the camera.

EXPERIMENT - After adding cytochalasin

I then used the standard that we used in lab to calculate what the length of each psuedopod was in micrometers. The

standard value that I used was 1.82micrometeres per pixel, and this was found by averaging the different numbers of number of micrometers per pixel of a ruler that the members of the class obtained when measuring the length per pixel in a picture of maginification 400, objective lens of 40X using a Nikon E200 light miscroscopeand a Spot Insight camera.

III. Results

	Width of Psuedopod(pixels)	Length of Pseudopod(pixels)
Control: Picture 1	74.673	112.641
Picture 10	142.618	130.614
Experiment: Picture 1	67.676	110.309
Picture 10	64	115.463

CONTROL

Picture#1: Length of pseudopod: 112.641 * 1.82microns/pixel
= 205 microns
= 0.205 mm
Width of Pseudopod: 74.673*1.82microns/pixel
= 135.9049microns
= 0.136mm
Picture 10(After 5 minutes): Length of pseudopod: 130.614 * 1.82microns/pixel
= 237.7175microns
= 0.238mm
Width of Pseudopod: 142.618*1.82microns/pixel
= 259.5648microns
= 0.260mm

EXPERIMENT - After adding cytochalasin

Picture #1: Length of pseudopod: 110.309 pixels*1.82micons/pixel
= 200.7624
= 0.200mm
Width of pseudopod: 67.676 pixels*1.82micons/pixel
= 123.173
= 0.123mm
Picture #10(After 5 minutes): Length of pseudopod: 115.463pixels*1.82micons/pixel
= 210.1427microns
= 0.210mm
Width of pseudopod: 64 pixels*1.82micons/pixel
= 116.48microns
= 0.116mm

	Width of Pseudopod(mm)	Length of Pseudopod(mm)
Control: Picture 1	0.136	0.205
Picture 10	0.260	0.238
Experiment:Picture 1	0.123	0.300
Picture 10	0.116	0.210

CONTROL PICTURES

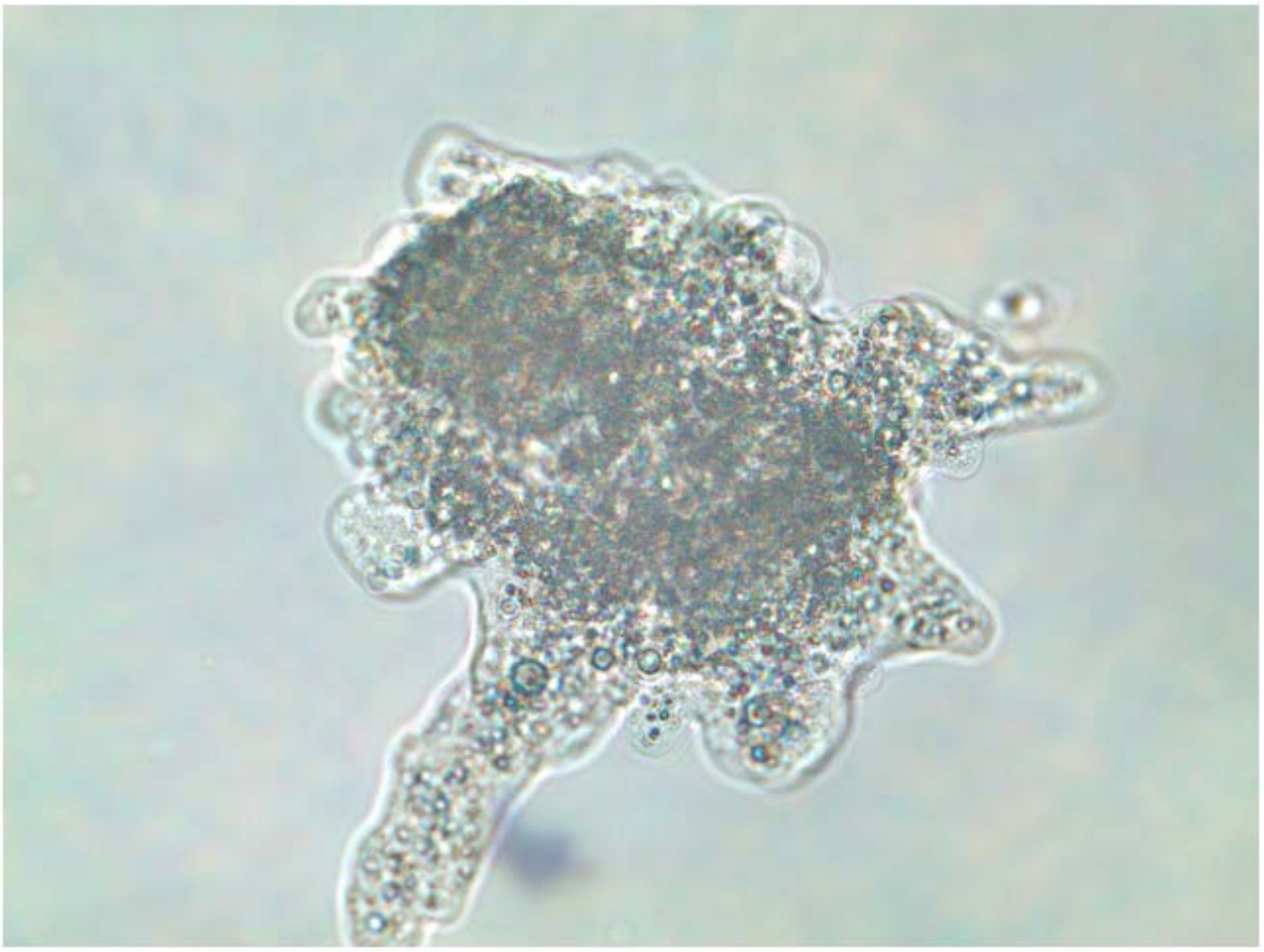

figure 1. This picture shows the amoeba at the start of sequential images. These sequentialimages were taken at 30 second intervals and they were 10 of them, so ittook 5 minutes between the first and last picture to be taken. It istaken at a magnification of 400 using the 40X objective of a Nikon E200 lightmicroscope and a Spot Insight camera. The sequential images were extractedfrom the sequential images file so as to obtain individual images that were part of the whole sequential images file with the use of a program Spot 3.5.5.

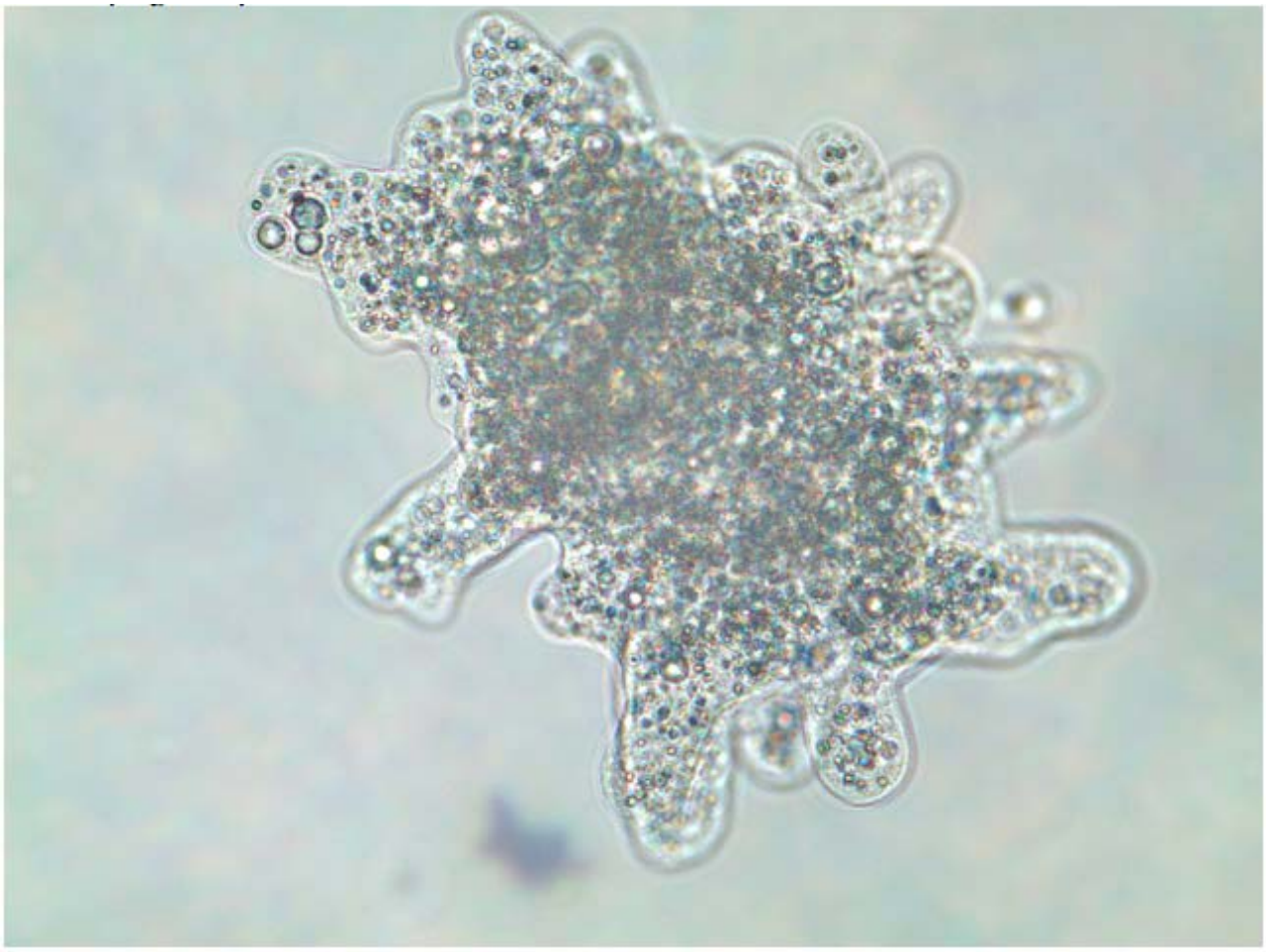

figure 2. This picture shows the amoeba at the end of sequential imagesthat I took. It took 5 minutes between the first and last picture to be taken.The picture is taken at a magnification of 400 using the 40X objective ofa Nikon E200 light microscope and a Spot Insight camera. The sequential images were extracted with the use of a program Spot 3.5.5. This amoebahas moved from its initial position. The pseudopods have changed shape and size accordingly.

EXPERIMENT PICTURES

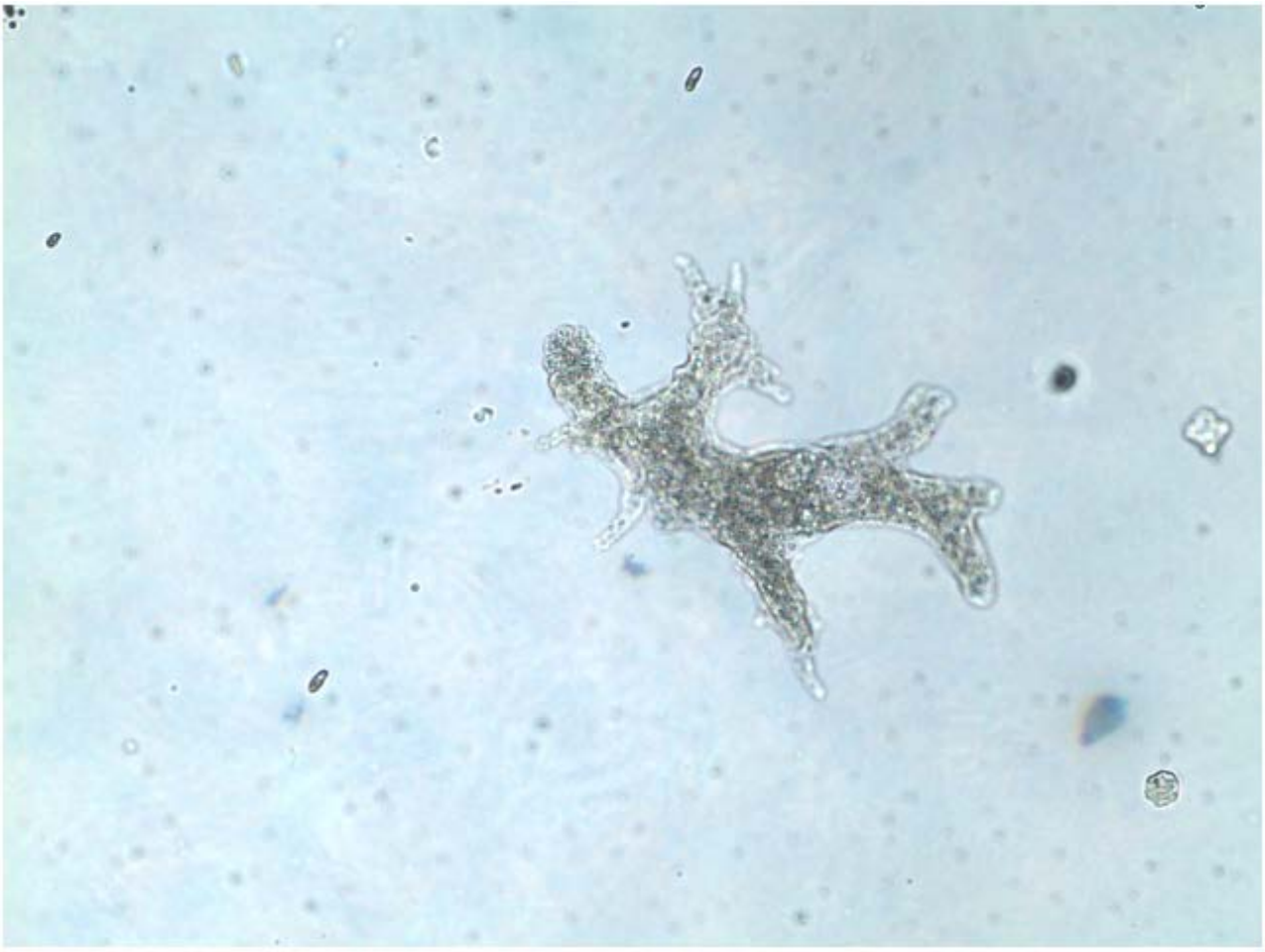

figure 3. This picture shows the amoeba at the start of sequential images that I took. Cytochalasin has been added to the amoeba to observe effecton the cell movement. These sequential images were taken at 30 second intervalsand there were 10 of them, so it took 5 minutes between the first and last picture to be taken. The picture is taken at a magnification of 100 usingthe 10X objective of a Nikon E200 light microscope and a Spot Insight camera.The sequential images were extracted with the use of a program Spot 3.5.5.

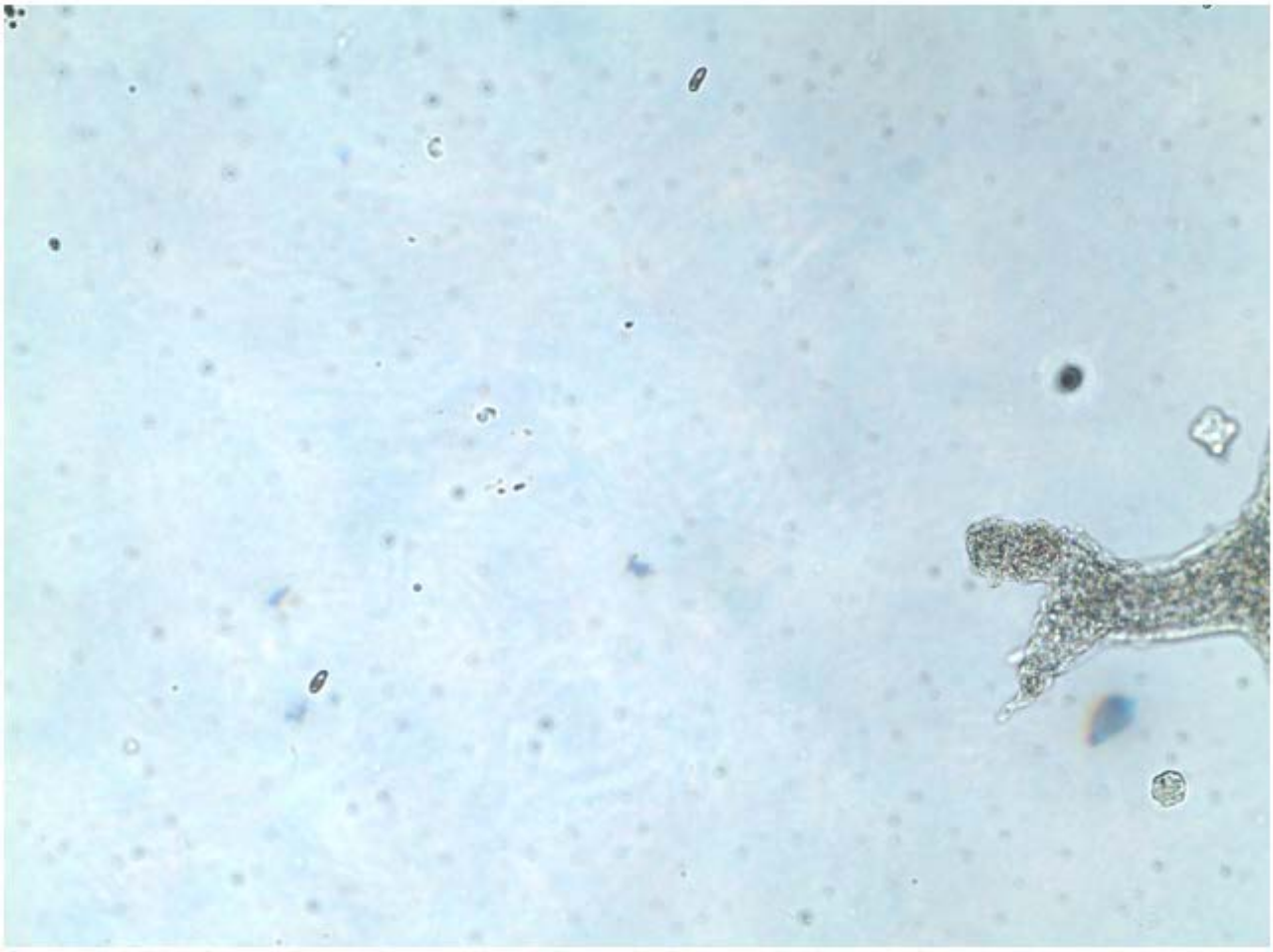

figure 4. This picture shows the amoeba at the end of sequential images that I took. It took 5 minutes between the first and last picture to be taken. The picture is taken at a magnification of 100 using the 10X objective of a Nikon E200 light microscope and a Spot Insight camera. The sequential images were extracted with the use of a program Spot 3.5.5. It is evident that this amoeba has moved from its initial position to another position, and the pseudopods have also changed in length and width at the same time.

IV. Discussion and Conclusions

From the pictures, it is evident that the cells are able to move from one place to another, and that the pseudopodia change shape. In the time lapse movie that we made for 5 minutes, the movement and change of shape and size of amoeba was more visible. Amoeba therefore have the ability to move from one place to another, and this is made possible by the cytoskeleton, of which actin plays a part. The results that I obtained mean that cytochalasin B increases the movement of amoeba. This is in contrast to the hypothesis that was I was testing. After adding cytochalasin B the movement of amoeba did not slow down as expected. Instead, the movement of amoeba increased within the same amount of time, 5 minutes. I did a few experiments to have consistent results and reduce the amount of errors that I would have possibly made when I added the drug, since the observations that I was making were not supporting the hypothesis that cytochalasin B slows down movement in amoeba by depolymerizing actin in the cytoplasm. This increased movement could be because the amoeba that I was observing after adding the drug were more mobile than the first ones that I had used for the control, since I did not use the same amoeba to test with the drug. If it is possible,it would produce less error in detecting the amount of movement by an amoeba,if I could use the same amoeba to look at movement before and after addingthe drug.

My partner, Daniel Stein, was looking at the effect of the drug nocodozoleand its effect on cellular movement also, and

nocodozole depolymerizes microtubules,which are also responsible for movement within the cell. He made similar observation that I made. After he added the drug to the amoeba proteus, the movement of the amoebae was increased. The concentration of the drug, cytochalasin B could also have not been enough to slow down the overall movement of amoeba. This drug could have slowed down parts of the cell, but did not affect the movement of the whole cell. It would be helpful for experiments in the future if I used an increased concentration of the drug, and observe whether this would have a significant effect on the movement of the cell.

V. Bibliography

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