The Effect of Decreasing Temperature on the Rate of Movement in *Amoeba proteus*

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(Image of *Amoeba proteus*. Scale-bar is 100 microns in length. Taken 11/19/2003 by Danielle M Kyes)

Introduction:

The cell behavior I studied was the movement of the *Amoeba proteus* across the plane of a room temperature glass slide (~23 degrees C) and across the plane of a glass slide after incubation in an ice bath (~0 degrees C). Movement, for the purpose of this experiment is defined as cytoplasmic movement that causes cells to acquire a new position or shape. *Amoeba protei* are relatively large protozoa that use mobile extensions of the cytoplasm called pseudopodia for movement and food capture (Gillis, R. 2003). The hypothesis was that amoebae kept incubated at 0 degrees C would move slower than the amoebae kept at room temperature. This is a temperature change of about 23 degrees C. Microtubules are filamentous structures found in nearly all eukaryotic cells, have been shown to depolymerize at 0 degrees C. This method is known as cold-induced depolymerization (Bokros et al 1993). Under the idea that microtubules might be responsible for cell movement within *Amoeba proteus*, as they are known to function in a variety of cellular movements that involves the polymerization and depolymerization of microtubules, and that microtubules depolymerize at cold temperatures, I think the rate of movement of the *Amoeba protei* will decrease when exposed to the cold temperature (Cooper & Hausman 2004).

The experiment involved obtaining *Amoeba proteus* from a culture dish and placing them on glass slides stored at room temperature, as well as glass slides that would be kept in on ice, and recording the movement in sequential images with a camera mounted on a light microscope, and then comparing the difference between the rate of movement between the amoebas at room temperature and the cold amoebas.

Materials and Methods:

A. Materials:

- *Amoeba proteus*
- Chip chamber glass slides and cover slides
- Ice in metal bowl
- VaLap
- Pipette
- SMZ660 Nikon Stereoscope
- Nikon Eclipse E400 Light microscope
- Spot Insight QE camera and Spot Advanced Software, version 3.5
- Macintosh G4 Computer
- Movement analysis grid (developed by Danielle Kyes, 11/19/03)

Metric ruler
Pencil

B. Methods:

Control:

The first step of experimentation was to find amoebae cells using a SMZ660 Nikon Stereoscope to locate cells in a culture dish and pipette as many amoebae as possible in the pipette. The cells were pipetted on to a chip chamber glass slide and covered with a glass cover slide. The sides of the cover slips were VaLaped to retain amoebae and their aqueous environment (pond water) and well as to prevent other objects from entering the chamber. The cells were located with a light microscope and a series of 30 sequential images were taken at 6-second intervals with Spot Insight Camera and Spot Advanced Software. This imaging was done with three different amoebas on room temperature slides (~25 degrees C).

Experimental:

Obtaining the *amoeba proteus* was the same for the experimental cells. However, the amoebae were placed on chip-chamber slides, covered with a glass cover slip, VaLaped, and put on ice (~0 degrees C) for 30 minutes before they were placed on the stage of the light microscope for observations. Like the control cells, a series of 30 sequential images were taken at 6-second intervals with Spot Insight Camera and Spot Advanced Software. The observations were done instantly after the slides had been taken of the ice as not to allow for drastic cooling before observing the movement.

Analysis:

Prior to analyzing cell movement, movement was defined as any cytoplasmic movement that caused cells to acquire a new position or shape. A grid was created to quantify movement in 8 directions. The grid lines were made at 0.5 cm intervals. The grid was taped to a flat-screen monitor in the Imaging Center for Undergraduate Collaboration. At 18-second intervals, the position and shape of the amoeba were marked on a new copy of the grid. The grids were analyzed to determine cm/sec of the amoeba. The amoeba was not moving at cm/sec, but the grid displays movement in these units. It is not necessary for the hypothesis that actual units are used to analyze movement as long as the method is consistent with each trial.

Results:

Both the control amoeba and cold amoeba were observed via brightfield light microscopy at 100 times magnification. Time-lapse imaging was used to capture the moving amoebae. The position and shape of each amoeba was recorded for 30 images at 6-second intervals. Because the amoebae commonly had moved out of the field of view within the 240 seconds, only images 3, 6, and 9 of each control amoeba and each cold amoeba were used for analysis. Movement in each of the 8 grid directions (denoted as A-H), for the three trials of the control and the three trials for the cold were then averaged. This results in a total net movement of controls and colds to be compared:

Average Movement of Control in arbitrary units								
A	B	C	D	E	F	G	H	Total
1.00	2.96	1.83	1.42	1.00	1.79	2.33	1.00	13.33

Average Movement of Cold in arbitrary units								
A	B	C	D	E	F	G	H	Total
0.75	0.81	0.83	0.50	0.50	1.42	1.29	0.79	6.89

The average total movement in the room temperature amoebae was 13.33 while the average total movement in the cold treated amoebae was 6.89. The total net movement can be converted to rate of movement cm/sec as the movement spanned over a time frame of 18 seconds:

Rate of Movement of Control	0.74 cm/sec
Rate of Movement of Cold	0.38 cm/sec

It may be important to note that each trial of the control and each trial of the cold had some variance in total net movement as to compare variance in movement between amoeba of the control and the variance in movement between amoeba of the cold to the difference in movement of the controls to the colds. The following table displays the movement of each trial:

Control 1									
Frame #	A	B	C	D	E	F	G	H	
3	13	11.5	7.5	3	2	1.25	3.5	10.25	
6	12	10.5	6.5	2	3.5	3	3.5	8.75	
9	10.5	9	6	3	4.5	4.75	6.25	7.5	Total
Average	1.25	1.25	1.25	1	1.25	1.75	1.375	1.375	10.5

Control 2									
Frame #	A	B	C	D	E	F	G	H	
3	12	3.75	17.25	11.5	0.25	-3.5	-4.75	1	
6	11	15	15	10.25	0.25	-0.5	0	1	
9	9.5	14	13.5	8	0.25	0.25	3	3.25	Total
Average	1.25	6.125	1.875	1.75	0	1.875	3.875	1.125	17.875

Control 3									
Frame #	A	B	C	D	E	F	G	H	
3	-2.5	2	5.75	7.25	16.5	15.75	10.5	0	
6	-1.75	3.5	8.25	9	15	13.75	8.75	0.75	
9	-1.5	5	10.5	10.25	13	12.25	7	1	Total
Average	0.5	1.5	2.375	1.5	1.75	1.75	1.75	0.5	11.625

Cold 1									
Frame #	A	B	C	D	E	F	G	H	
3	5.5	4.5	1.75	10	12.5	10.25	6	3.5	
6	3.75	3.75	2.75	11.5	13.5	10	5.5	1.25	
9	4	4.25	3.5	12.5	13.5	8	3.5	1	Total
Average	1	0.625	0.875	1.25	0.5	1.125	1.75	1.25	8.375

Cold 2									
Frame #	A	B	C	D	E	F	G	H	
3	10	9	2.75	1.5	0.5	4.5	5.5	8	
6	11.25	9.5	3.75	1.5	0.5	0.75	4.25	7.75	
9	12.5	11	5	3.5	0.75	0	2.75	6	Total
Average	1.25	1	1.125	1	0.125	2.25	1.375	1	6.875

Cold 3								
Frame #	A	B	C	D	E	F	G	H

	3	5.5	7	5	5.5	8.25	7.25	3.5	0.75	
	6	5.5	7	4.75	6	10	8.25	3.75	0.75	
	9	5.5	7	5.5	7.25	10	9	5	0.5	Total
Average		0	0	0.5	1.375	0.875	0.875	0.75	0.125	4.5

Figure 1: Each box is a trial of either a control or cold amoeba and it's position at frames 3, 6, and 9. The total amount the amoeba moved in each trial is stated to the right of each box. The units of movement are irrelevant to the focus of the experiment and should be considered simply "units of movement." The average movement from frame 3 to 6 and from 6 to 9 determined total movement in each of the 8 directions noted as A-H. The sum of these totals gives the final total of movement.

The variance of net movement between the controls is a range of 10.5-17.875 arbitrary units, while the variance of net movement between the colds is a range of 4.5 to 8.375.

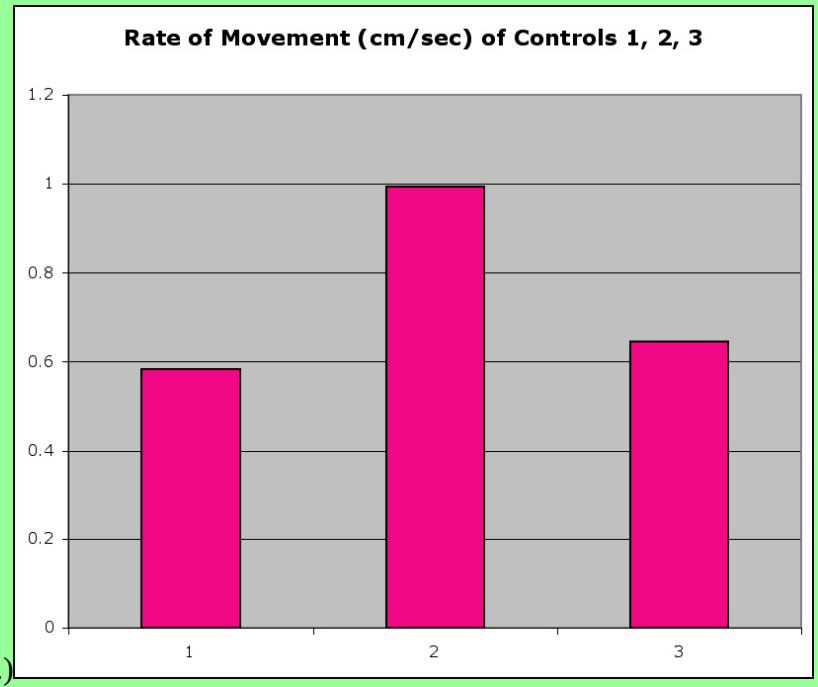
The total movement as displayed in Figure 1 can be converted to units of cm/s. The time interval is 18 seconds. Table 1 displays results in cm/sec:

Control 1	.583 cm/sec
Control 2	.993 cm/sec
Control 3	.646 cm/sec
Cold 1	.465 cm/sec
Cold 2	.382 cm/sec
Cold 3	.250 cm/sec

Table 1. Rate of movement of control and cold *Amoeba proteus*

The results show that when the amoebas were cold-treated, the rate of movement decreases. The following figures are of the rate of movement for the control amoeba and cold amoeba:

a.)



b.)

Introduction:

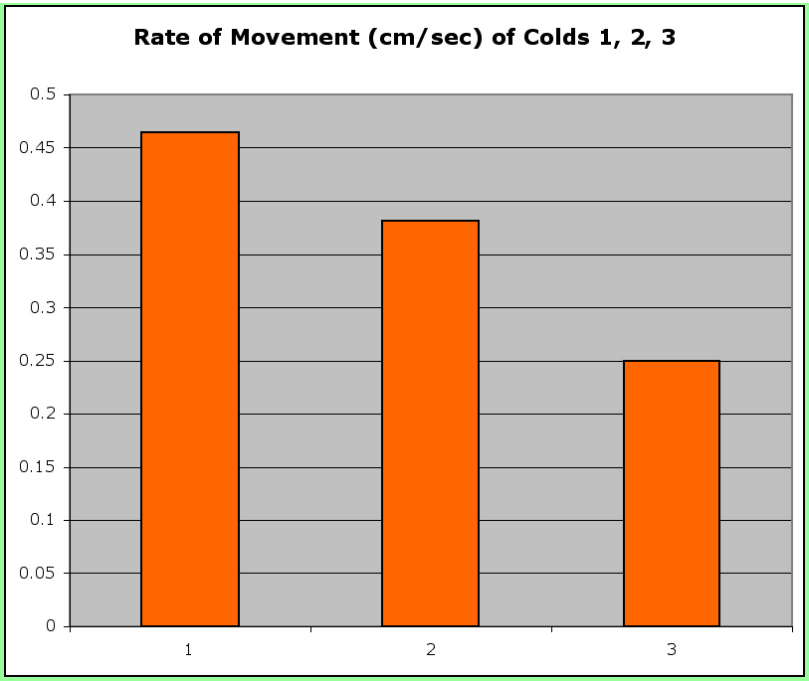


Figure 2: (a) Graph of the rate of movement of the 3 controls in representation. (b) Graph of the rate of movement of the 3 cold amoebas.

When comparing the total movement of the controls and cold amoeba, it is clear that the rate of movement of the control cells is greater than that of the cold amoeba:

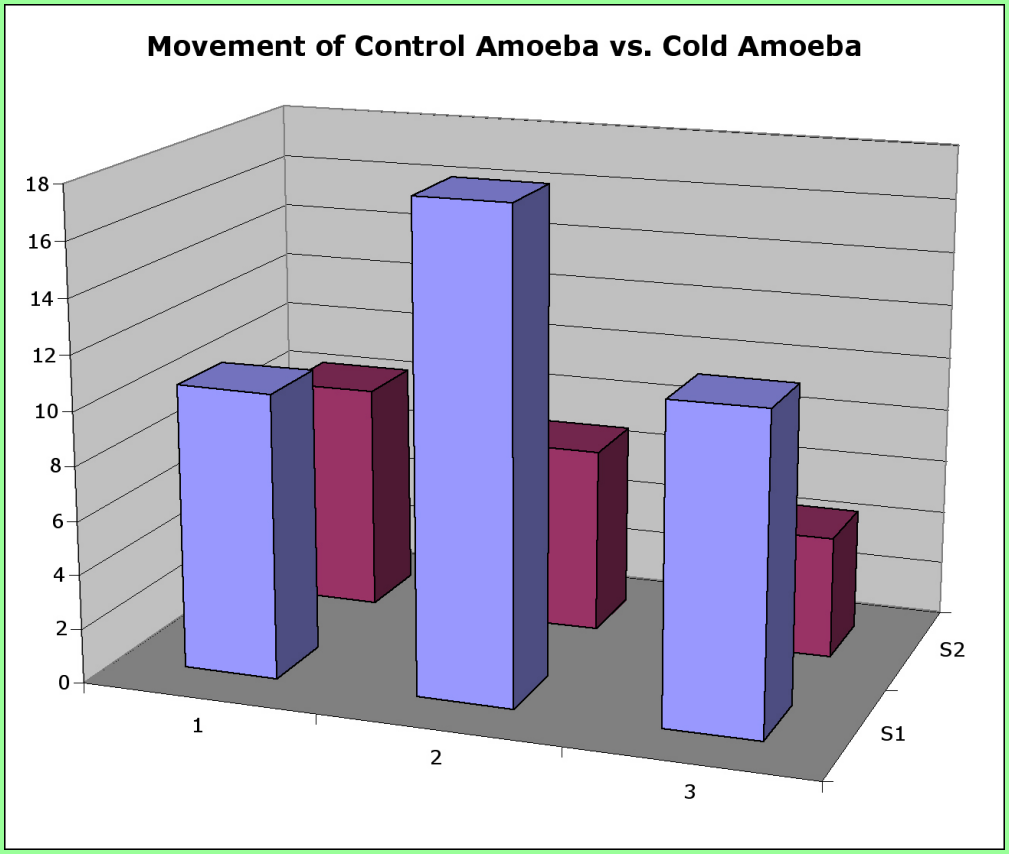


Figure 3: The blue rectangular solids represent the total movement of each of the 3 control amoebas and the magenta rectangular cubes represent the total movement of each of the 3 cold amoebas.

Discussion and Conclusions:

The results of comparing the movement of amoebas at room temperature (~23 degrees C), and amoebas at freezing temperature (~0 degrees C) show that the cold temperature affects the rate of movement of *Amoeba proteus*. These results support my hypothesis that decreasing the temperature would slow the rate of movement. However, the hypothesis stated the rate of movement would decrease because of microtubule depolymerization. This hypothesis is neither supported or refuted, but undetermined with the results obtained. The significance of this result is that further experimentation is needed to test this hypothesis. Experimentation must be microtubule specific and the methods used to generate the above results cannot determine that the rate of movement of amoebae when exposed to freezing temperatures is necessarily directly related to microtubule depolymerization.

It was important to see that while there was variance in the movement between the trials of each the control and the cold trials, the variance in the range did not overlap. That is, the entire range of movement of the control was higher than the entire range of movement of the cold. This provides further support to the hypothesis that the rate of movement of *Amoeba proteus* is lowered when kept at 0 degrees C.

While the results show reduced cell movement, it undetermined to exactly why the cold affects the movement of the *Amoeba proteus*. Speculation of the possibilities involve what is happening to the cell at 0 degrees C. It is the cytoskeleton of the cells that is responsible for movement, thus there must be a change in the cytoskeleton of the cells when cold. Cytoskeleton is composed of actin filaments, intermediate filaments, and microtubules. The change in the cytoskeleton therefore is directly related to a change of one, all three, or any combination of the three (Cooper & Hausman 2004). Determining which are responsible for the change in cytoskeleton, and thus the change of movement would involve further experimentation.

Sources of Error:

Sources of error in this experimentation come from the effect of rounding and averaging measurements. The grid for analysis is marked in 0.5 cm intervals and the marked positions were rounded to the nearest 0.25 of a cm. Also averaging was used to quantify movement. This gives a better overall result, but does not reveal the exact rate of movement of a particular amoeba. Maintaining the cold (~0 degrees C) was manageable as the sequential images were taken immediately after the slide was taken off the slide and the examined sequential images spanned over 54 seconds (3 images taken 18 seconds apart).

Collaborator's Results:

My student collaborator Rob Borkowski conducted a similar experiment to the test the rate of movement of the *Amoeba proteus*. Instead of testing the rate of decreasing temperature, Borkowski tested the effects of the drug Nocodozole, a microtubule depolymerizing drug, on the rate of movement. The results show that Nocodozole does slow the rate of movement in the *Amoeba proteus*. Because the depolymerization of microtubules slow the rate of movement in the cells, this supports the possibility that freezing temperatures initiate depolymerizing of microtubules. Again, further experimentation is needed to support this hypothesis.

Reformations:

To refine this experiment, more data of movement would be useful for better accuracy. The experiment would benefit from testing movement over a greater number frames and with more than three trials for each the control and the cold.

Further Experimentation:

While this experiment was conclusive in the degree that freezing temperatures slow the rate of movement in the *Amoeba proteus*. However, whether or not the cold temperature affected depolymerization was not concluded within this experiment. Further experimentation would involve using fluorescence microscopy to fluoresce the microtubules in a cell at room temperature and also the microtubules of cells incubating in ice.

Also,

Bibliography:

1.) Bokros, Carol L. et al. 1993. Characterization of the reversible taxol-induced polymerization of plant tubulin into microtubules. *Biochemistry* 32: 3437-47.

2.) Cooper and Hausman 2004. *The Cell A Molecular Approach*, 3rd ed. ASM Press, Washington D.C. pp. 435, 463.

3.) Zoo Lab, 2003. *Amoeba proteus*. http://bioweb.uwlax.edu/zoolab/Table_of_Contents/Lab-2b/Amoeba/amoeba.htm (November 24, 2003).

In collaboration with Rob Borkowski, Wheaton College, class of 2006.