Bio254 Home

Evidence that Mercury Decreases the Area of Aster Formation in *Lytechinus variegatus* Embryos During the Cleavage Stage

Cali McGinn

Web Posted on 12-2-04

Introduction	Materials and Methods	Results	Discussion and Conclusions	References

I. Introduction

Adult organisms are physically different from each other, but their initial developmental processes are very much alike. Fertilization of an egg by a sperm is the first step in the developmental process in sexually reproducing animals. The development of multicellular organisms then continues with the process called cleavage. During this process, the cell replicates its DNA and aligns the chromosomes along the metaphase plate. Then the cell will form asters, an array of microtubules that radiate outward from the spindle poles. The asters help support the function of the spindle fibers in separating the chromosomes. Cleavage is stimulated by the activation of the mitosis promoting factor and involves a sequence of mitotic divisions that divide up the cell's cytoplasm. Mitosis results in the formation of many smaller, nucleated cells. The cleavage process ceases after the embryo has achieved a new equilibrium between the nucleus and the cytoplasm. (Gilbert, 2000) Development will progress until the animal has matured into the adult stage.

Sea urchins are marine invertebrates that live on the sea floor. They are globular animals with hard, calcareous shells that are armed with spines. Sea urchins are five-fold radially symmetric invertebrates that are indirect developers and they make use of set-aside cells. ("Sea Urchins", 1995) Although there is much debate about the function of set-aside cells, it is believed that most of the large parts of the adult sea urchin are derived from the set-aside cells. (Wilt, 2004) Indirect developing organisms have an intermediate larva stage in their developmental process that follows embryogenesis, which begins with the cleavage process. A sea urchin larva is called the pluteus and has many morphological differences from the adult. (Wilt, 2004) The pluteus will then undergo metamorphosis and will develop into an adult sea urchin.

In this experiment, I studied the cleavage process of sea urchin embryos. This process is very significant to study because it is the initial differentiation process of development. If cleavage either doesn't occur or occurs abnormally, the continued development of the organism will be disrupted. This could lead to a deformed adult or, in a more extreme case of abnormality, a dead embryo.

Mercury is a toxic pollutant that has many harmful properties. Studies have shown that the exposure of mercury to an organism can have detrimental effects on its development. One study in particular found that prenatal mercury intoxication of rats disrupted the effects of dopamine on flash visual evoked potentials in the brain. (Herba E. et al., 2004) On a cellular level, mercury can also have damaging effects on the mitochondrion's functions, the production of proteins, and the process of mitosis. Mercury impairs the process of cell division, differential and migration because it binds to the thiols of the tubulin proteins, which make up the microtubules. ("Mercury", 2000) Therefore, it is believed that mercury may affect the developmental process.

In this study, I tested the hypothesis that the area of the aster formation will decrease within the sea urchin embryos as the amount of mercury that the eggs were exposed to increases. I fertilized sea urchin eggs that were in various concentrations of mercury with normal sea urchin sperm and observed the development of the sea urchin embryos. Then I compared the measured areas and made conclusions about the effect of mercury on the rate of mitosis during the cleavage process.

Back to the Top

II. Materials and Methods

MATERIALS

For this experiment, *Lytechinus variegatus* sea urchins and filtered natural sea water were used. Two standards of mercury with concentrations of 5ng/mL mercury and 20ng/mL mercury were also used. In order to perform the experiment, sterile Pasture pipettes, an adjustable micropipette, tweezers, slides, cover slips, Petri dishes, a permanent marker, masking tape and double-sided Scotch tape were used. For equipment, a Nikon Eclipse E200 Microscope, a Spot Insight QE camera and a clock were used. Particular computers programs such as Spot Advanced, Adobe Photoshop, Image J and Microsoft Excel were used to collect and analyze our data.

METHODS

Overall, each of the two trials for this experiment took about four and a half hours to complete. Only one control was necessary for this experiment. The control condition was sea urchin eggs that were not exposed to any mercury. This control was very appropriate for the experiment because it provided a set of standard results that were used to compare the results of the experimental conditions against. The control results suggested the natural results of the studied process without any manipulations.

PREPARING CONDITIONS

Two different standards of mercury in .5% nitric acid, 5ng/mL and 20ng/mL, were gathered for this experiment. (Benoit, 2004) Four Petri dishes and covers were then labeled control, 5ng/L, 20ng/L or 100ng/L using a permanent marker. Next, a female Lytechinus variegates sea urchin was shed into filtered natural sea water at room temperature. Immediately after shedding, a portion of the egg solution was collected by sterile Pasture pipettes and divided, equally, into 4 Petri dishes. If necessary, more filtered natural sea water was added to each Petri dish so that each dish contained 10mL of solution. Then 10µL of the 5ng/mL of mercury solution were added, using an adjustable micropipette, to the Petri dish labeled 5ng/L to obtain that final concentration of mercury in the dish. Next 10µL of the 20ng/mL of mercury solution were added, using an adjustable micropipette, to the Petri dish labeled 20ng/L to obtain that final concentration of mercury in the dish. Then 50µL of the 20ng/mL of mercury solution were added, using an adjustable micropipette, to the Petri dish labeled 100ng/L to obtain that final concentration of mercury in the dish. The sea urchin eggs remained in the mercury solution for an hour. During that time nine slides were prepared using double sided scotch tape. Two pieces of tape were placed on each slide about 2 centimeters apart.

MEASUREMENTS

After the first hour, small samples of eggs from each of the conditions were placed on four different prepared slides between two tape spaces. The cover slips were applied to the slides so that the two side edges of the cover slips were placed on top of the two pieces of tape. Then pieces of masking tape were placed on the edges of the slides so that the slides could be appropriately labeled, using the permanent marker, indicating which condition the eggs were from. Then the eggs from each condition were observed under an E200 microscope at 40X. Pictures of the eggs were taken with the Spot camera and the Spot Advanced computer program. Once this was completed, drops of eggs from each of the conditions were placed on the edge of four more slides, in between the two pieces of tape. The slides were then labeled with a piece of masking tape, which indicated the condition of the eggs. Then some sperm from a male sea urchin was collected. During the first trial, an activated suspension of sperm was collected from a gonad of a male sea urchin that had been explanted, at room temperature, into a Petri dish with filtered natural sea water and then had been squeezed with tweezers for the release of sperm. During the second trial, an amount of dry sperm was collected from a male sea urchin and then activated by the addition of filtered natural sea water. A drop of the sperm suspension was placed on a slide and observed under the microscope at 40X. Then, drops of sperm were placed on each of the slides on the opposite side of the egg droplets. Cover slips were then placed on top of the slides so that the two droplets would merge together. The times of the individual mergers were observed from the clock and recorded on the pieces of masking tape on the slides. Then each slide was observed under the microscope at 40X to see if any of the eggs were undergoing fertilization envelope lift off, which indicated that fertilization had occurred. Pictures of the eggs in each condition were taken. Then the slides sat on a counter at room temperature for about an hour, during which time the control embryos were expected to have begun to undergo their first cleavage. (Morris, 2004) At an hour after fertilization, the embryos of the different conditions were examined under the microscope at 40X. Pictures were taken of different embryos within each condition. At an hour and a half after fertilization, when the embryos of the

normal condition were expected to be undergoing their second cleavage, all the embryos of the different conditions were examined under the microscope again at 40X. (Morris, 2004) More pictures were taken of the embryos in each condition.

DATA ANALYSIS

The pictures that were taken at about 1 minute, 1 hour and 1.5 hours after fertilization, during the two trials, were pooled together. Only the pictures that showed clear aster formation and clear boundaries between the aster and the rest of the cytoplasm of the embryo were used in the data collection. The contrast of each picture chosen for data collection was changed to +40 using the Adobe Photoshop computer program. Then the area of the aster was measured using the Image J computer program. The largest area of the aster was chosen to be measured. The boundary between the aster and the rest of the cytoplasm was defined as the furthest point away from the center of the aster that the radial pattern of microtubules existed within the cells. The data collected for the area of the asters were in terms of pixels at 40X. In order to convert the measurement to pixels/ square millimeters, a picture of a ruler with millimeter markings was taken under the same microscope used during the experiment at 4X and the number of pixels in a square millimeter was measured using the Image J computer program. Then that measurement was converted to pixels/ square micrometers at 40X so that all the data could be converted in the same way. Lastly, the data for each condition were averaged together so that a single value accurately represented the data. These data were then entered into a worksheet in the Microsoft Excel computer program and a plot graph was created for the data.

Back to the Top

III. Results

The non-manipulated eggs and the sperm were observed separately, prior to the start of the experiment, to make sure that they were both healthy. Both the eggs and the sperm looked and acted as expected in their natural environment.

The sea urchin embryos within the different conditions varied from each other in a couple of ways. The embryos of different conditions varied from each other in terms of the stages of mitosis that the embryos were undergoing at the time they were observed. Among all the conditions, there was also, at least, a slight difference in the average size and in the definition of the aster formation in the cells of the embryos.

The cells didn't divide similarly in some of the conditions. The cells in the control and 5ng/L conditions were at identical stages of development throughout the experiment. The cells in the 20ng/L condition divided a little slower then the control and 5ng/L cells. The cells in the 100ng/L condition divided much slower in comparison to the cells in the other conditions and even failed to divide one hour after fertilization.

		Condition			
		0 ng/L (control)	5 ng/L	20 ng/L	100 ng/L
Developmental Observations of Cells at the Given Time After Fertilization	~ 1 minute	large fertilization envelope	large fertilization envelope	medium fertilization envelope	medium fertilization Envelope
	~ 1 hour	telophase	telophase	early telophase	condensed DNA
	~1.25 hour	metaphase	metaphase	metaphase	Telophase
	~ 1.5 hour	2nd telophase	N/A	N/A	N/A

FIGURE 1. This table represents the average developmental stage the cells were at the given time after the initial fertilization. For 0 ng/L, n = 6. For 5 ng/L, 20 ng/L and 100 ng/L, n = 2.

The cells also differed from each other in their aster formation development. They also varied in terms of the definition of the radial pattern of the aster.
In the control condition an hour after fertilization, the radial pattern of microtubules forming the asters in the cells was defined. The asters of the cells were very well developed and were distinguishable from the rest of the cytoplasm. Also, the areas of the asters in these cells were very large.

In the 5ng/L condition an hour after fertilization, the radial pattern of microtubules forming the asters in the cells was defined. The asters of the cells were developed and fairly large. The areas of the asters were very close to the areas of the asters in the control condition.

In the 20ng/L condition an hour after fertilization, the radial pattern of microtubules forming the asters in the cells was observable, but the pattern was not as clear as in the two previous conditions. The boundary between the aster and the rest of the cytoplasm was barely distinguishable. The areas of the asters of the cells were smaller than the areas of the asters for both the control and the 5ng/L condition.

In the 100ng/L condition an hour after fertilization, the small aster formations were indicated by the radial pattern of microtubules. In most of the cells, there were two aster centers of radiation but the boundary between the two asters was indistinguishable. Because the boundary was unclear, the two asters were measured together as one larger aster.

In the 100ng/L condition an hour and fifteen minutes after fertilization, the radial pattern of the asters was not as defined as the pattern of the asters in the other conditions. The asters in these cells were also not well developed. The areas of the asters in this condition, at this time after fertilization, were the smallest areas among the other conditions.

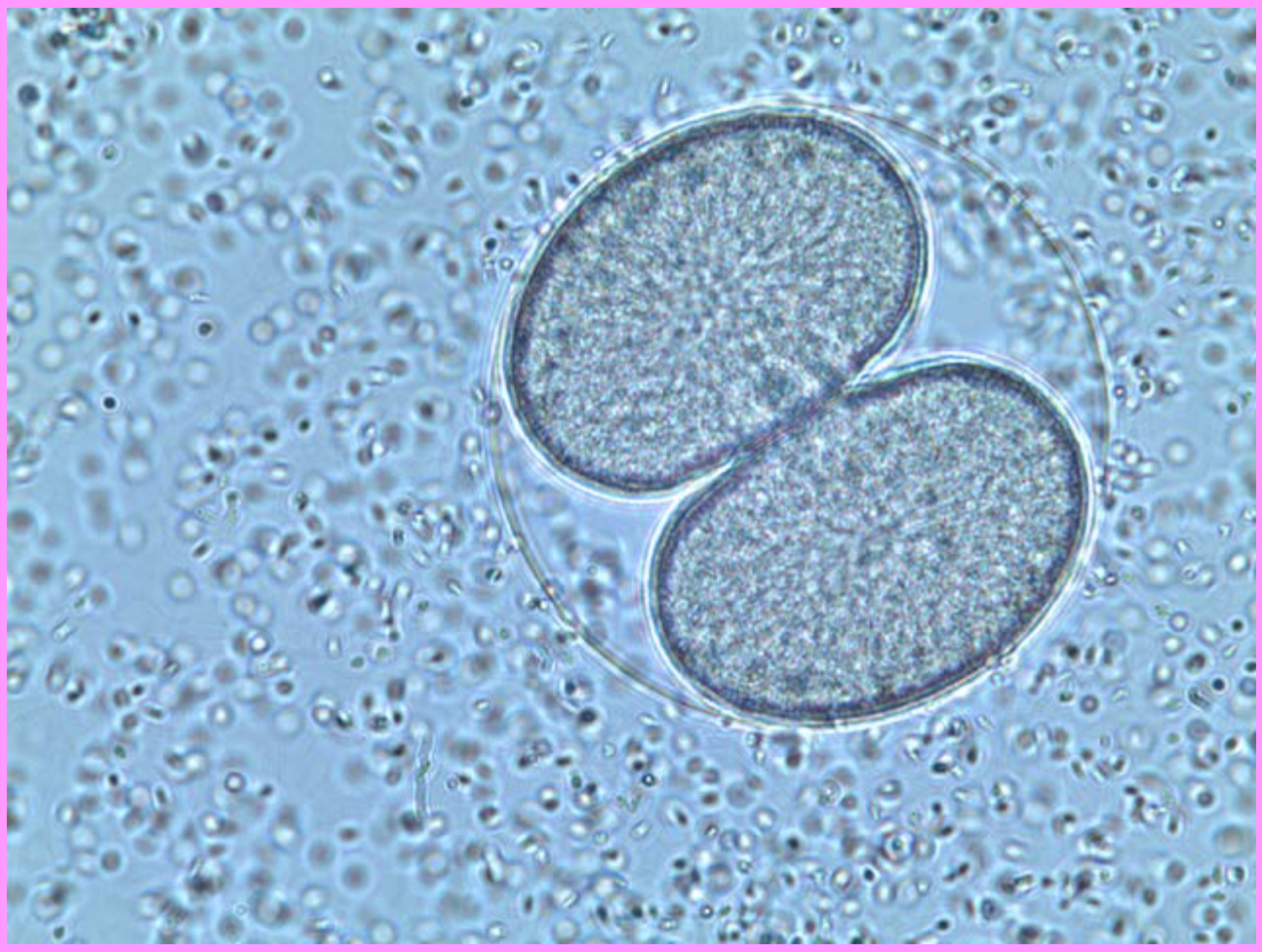

FIGURE 2. This is a picture of a sea urchin embryo from the control condition that was taken approximately 1 hour after the fertilization of a healthy egg by a healthy sperm. Notice how large the asters and how they organize the cytoplasm out to the cell periphery.

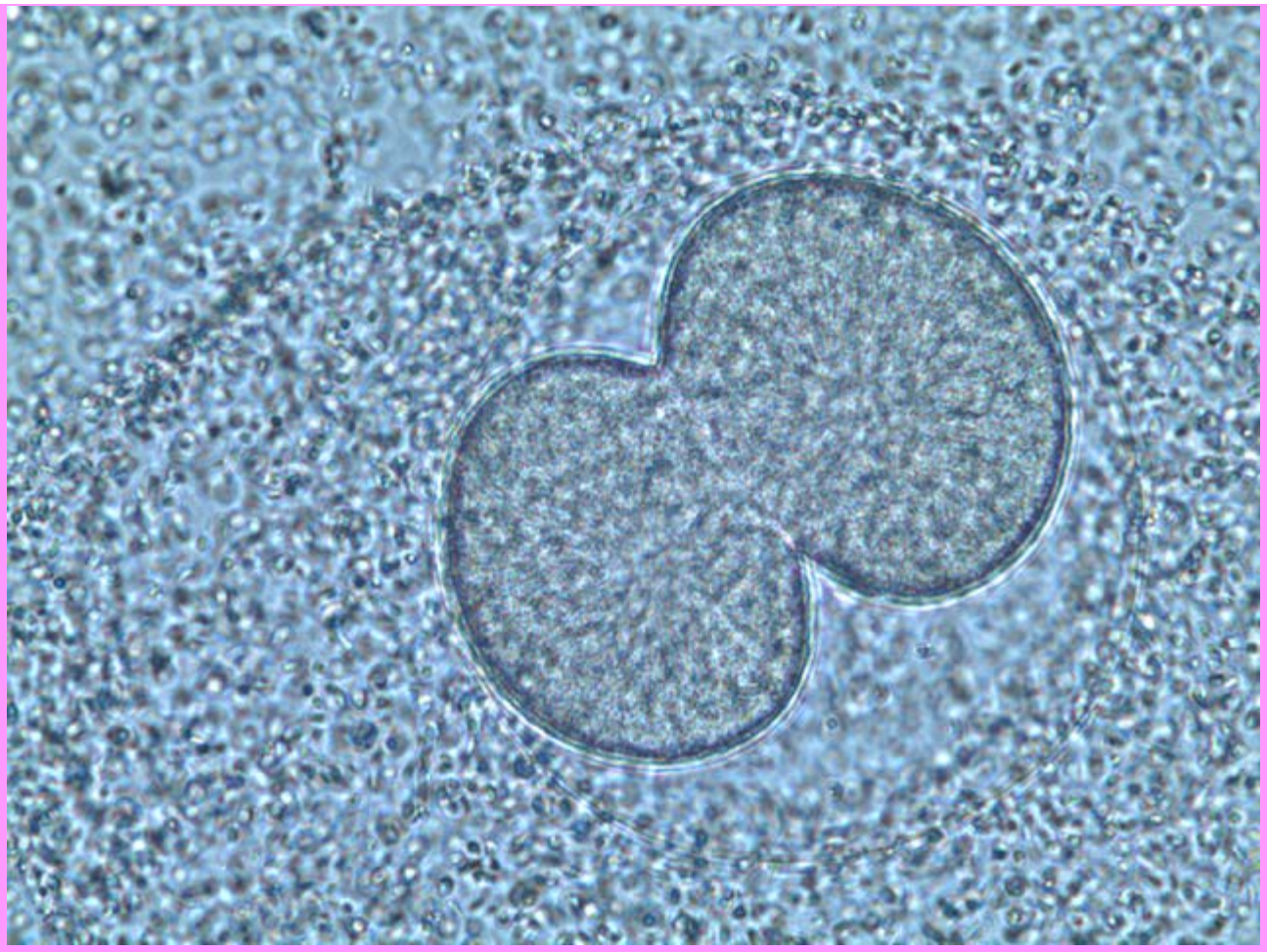

FIGURE 3. This picture of a sea urchin embryo was taken approximately 1 hour after the fertilization of an egg that was exposed to 5 ng/L of mercury by a healthy sperm. Notice how large the asters and how they organize the cytoplasm out to the cell periphery. The cells look very similar to the cells in the control condition.

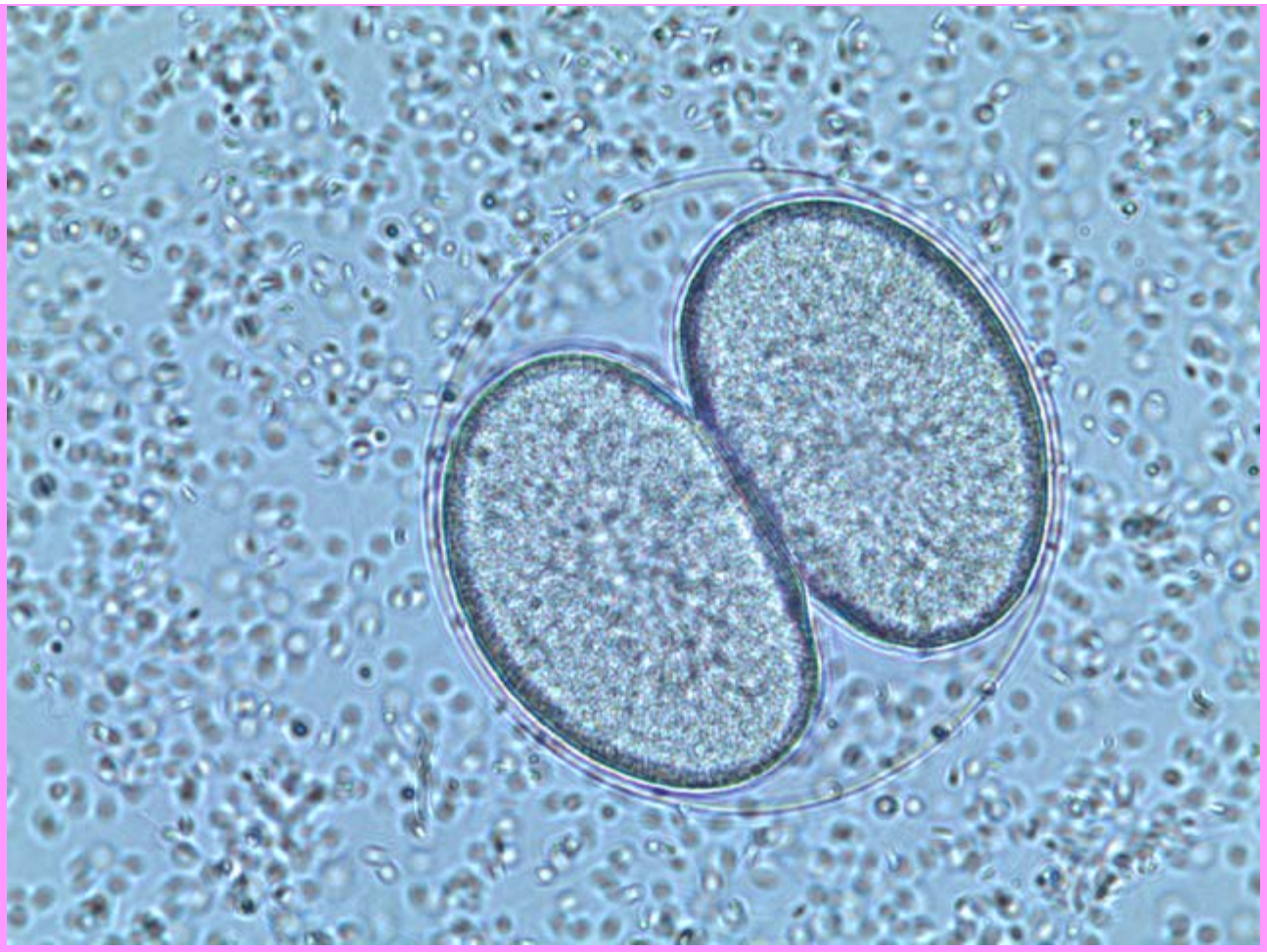

FIGURE 4. This picture of a sea urchin embryo was taken approximately 1 hour after the fertilization of an egg that was exposed to 20 ng/L of mercury by a healthy sperm. Notice the medium size of the asters and how they don't organize the cytoplasm very far out to the cell periphery. This embryo looks slightly less developed than the embryos in the control and 5ng/L conditions.

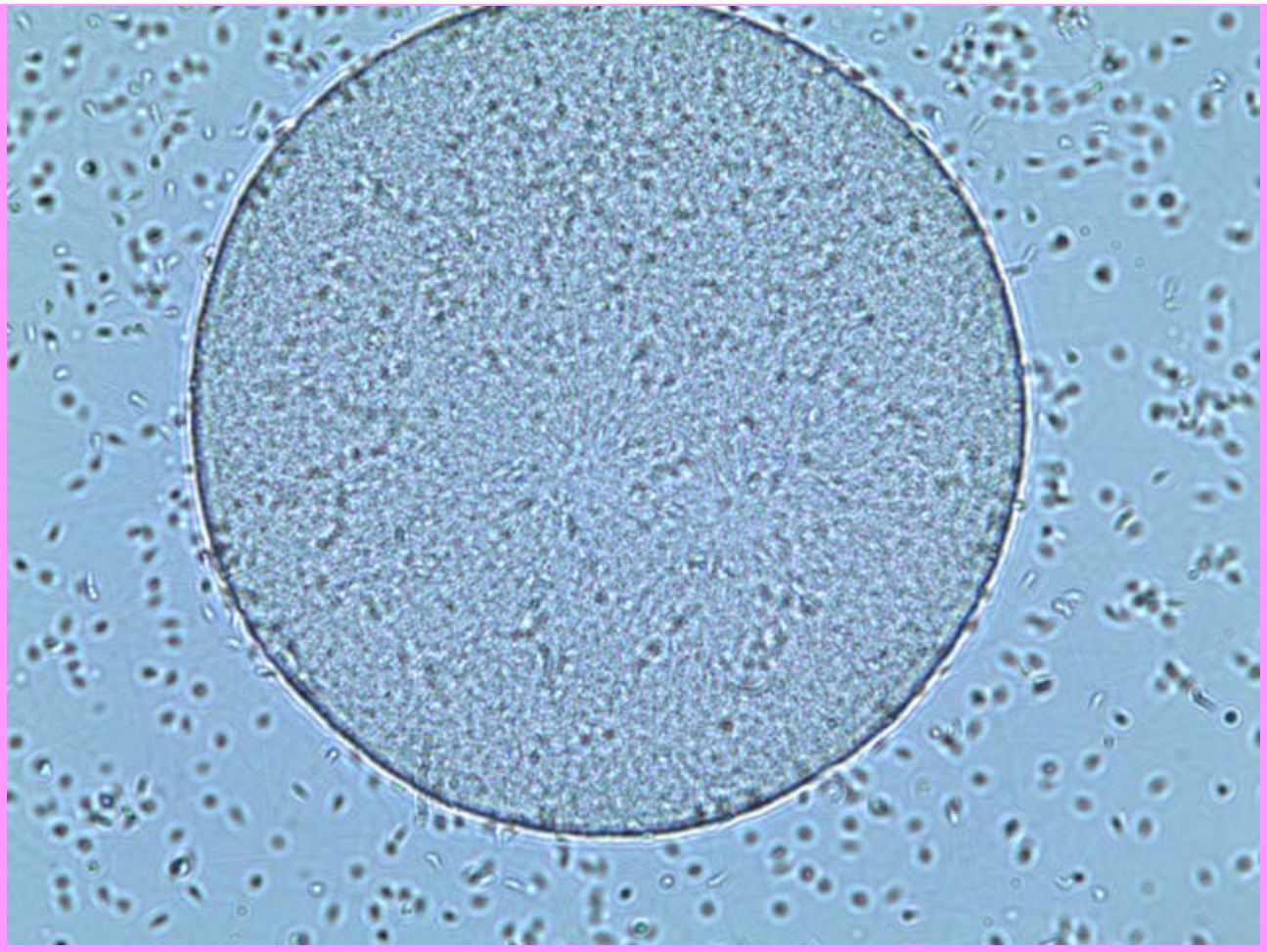

FIGURE 5. This picture of a sea urchin embryo was taken approximately 1 hour after the fertilization of an egg that was exposed to 100 ng/L of mercury by a healthy sperm. Notice how the two aster centers overlap and are position in the center of the cell. This embryo is less developed compared to the embryos in all the other conditions.

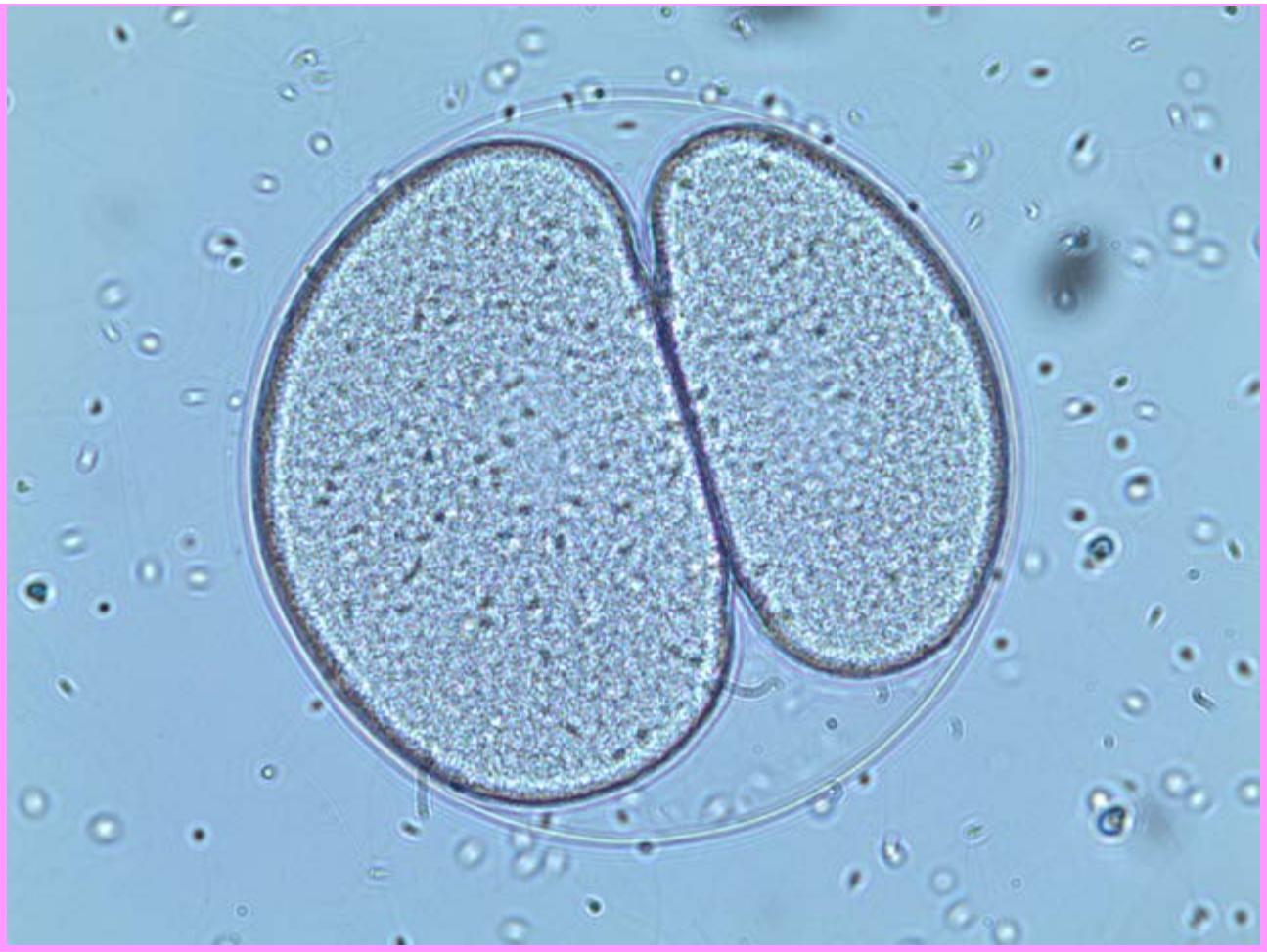

FIGURE 6. This picture of a sea urchin embryo was taken at about 1.25 hours after the fertilization of an egg that was exposed to 100 ng/L of mercury by a healthy sperm. Notice how small the asters and how they are positioned in the periphery of the cell rather than in the center of the cell.

The graph of the pooled data of aster area showed that, as the concentration of mercury that the eggs were exposed to prior to fertilization increased, the area of the aster within each cell decreased. The embryos of the control condition had the largest aster area. The embryos of the 5 ng/L mercury condition had aster areas that were very close in size to the control condition. The aster areas of the 20 ng/L and 100 ng/L condition were significantly less than the areas of the control condition.

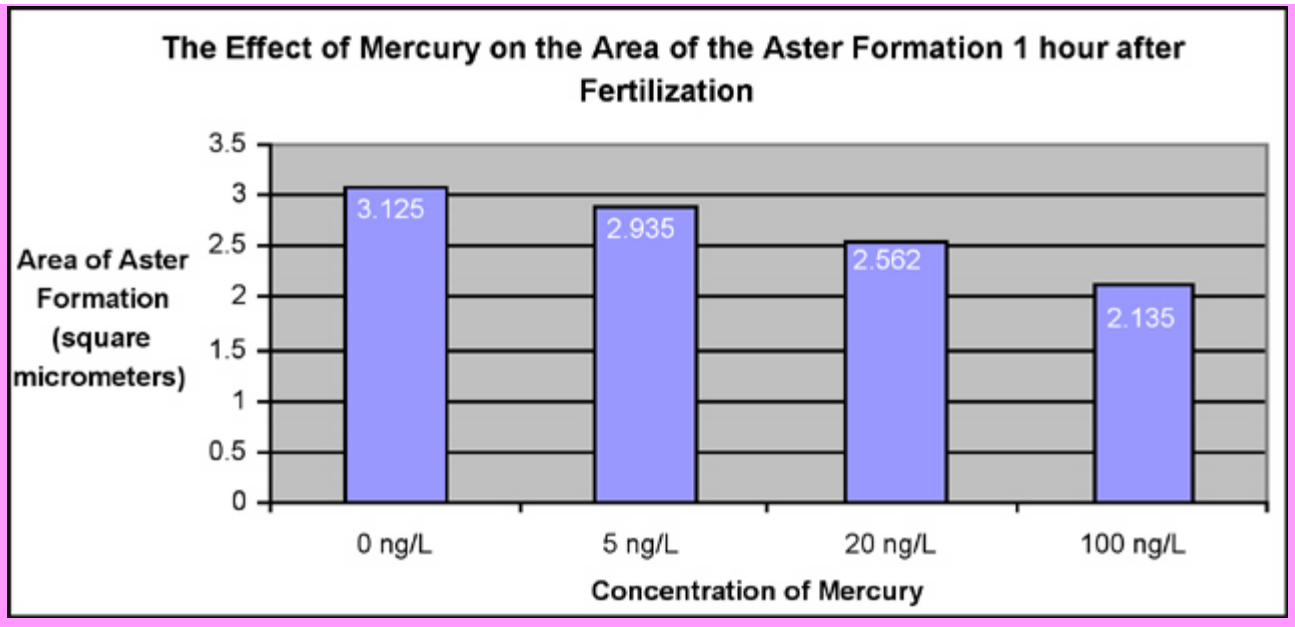

FIGURE 7. This graph shows the decrease in the average area of the aster formation of 1 hour embryos as the concentrations of the mercury the eggs were exposed to increase. For 0 ng/L, n = 6. For 5 ng/L, 20 ng/L and 100 ng/L, n = 2.

Back to the Top

IV. Discussion and Conclusions

The results from this experiment support the hypothesis that the area of the aster formation decreases within the sea urchin embryos as the amount of mercury that the eggs were exposed to increases. By exposing the sea urchin eggs to different concentrations of mercury and then fertilizing the eggs with healthy sperm, we were able to test the effects of the mercury exposure on the embryos.

From our results, it was clear that our experiment was successful. Because fertilization envelope lift off was observed in all of the eggs in the control condition, we concluded that all of the eggs had been fertilized. The high rate of fertilization in the control condition indicated that the eggs used for this experiment were all initially healthy. This allowed us to deduce that any deviation from the results of the control condition that were observed in the experimental conditions occurred as the result of the mercury exposure.

Fertilization envelope lift off was observed immediately after fertilization in nearly all of the cells in the other conditions as well. This indicated that the eggs in the experimental conditions were fertilized. From these results, we concluded that the exposure of mercury to the eggs did not disrupt the fertilization process.

In addition, the embryos in the control condition were undergoing mitosis and dividing at about one hour after fertilization. The embryos also divided once more during the next half an hour after the first division. This observation was expected because sea urchin embryos undergo their first

mitotic division an hour after fertilization and then continue to divide every half hour afterwards. (Morris, 2004)

Most of the cells of the embryos in the control condition had large asters an hour after fertilization. Since asters help support the spindle fibers in separating the chromosomes, which is a vital part of mitosis, their formation can be used as a reference for the mitotic rate of the cells. Therefore, we concluded that the cells were in the very advanced stages of mitosis because their asters were so large and well developed.

Even though there was a difference in average area of the asters between the 5ng/L condition and the control condition, we concluded that the difference was insignificant because it was so small. Since the asters were as similarly defined as the asters in the control condition, we concluded that these cells were also in the very advanced stages of mitosis. We also concluded that the exposure of 5 ng/mL of mercury to eggs did not affect the aster formation or the mitotic rate of the sea urchin embryos during the first hour after fertilization.

Since the embryos in the 20ng/L condition were asters were less advanced, we concluded that these cells were not as advanced in the mitotic process as the control condition cells. We also concluded that the exposure of 20 ng/mL of mercury to the eggs had a slight, negative affect on both the aster formation and the mitotic rate of the sea urchin embryos during the first hour after fertilization.

The embryos in the 100 ng/mL condition were very different from the cells in the control condition in terms of their aster size and definition and their mitotic rate. Therefore, we also concluded that the exposure of 100 ng/mL of mercury to eggs had a significant, negative affect on the aster formation and the mitotic rate of the sea urchin embryos during the first hour and fifteen minutes after fertilization.

Overall, our compiled data provided evidence that mercury decreases the area of the aster formation in Lytechinus variegatus embryos during the cleavage stage of their development. Our results were valid and accurate in respect to this particular experiment that we conducted. The results from this experiment supported our initial hypothesis.

However, there were a few sources of error in our experiment. The recorded time of the pictures is not completely accurate. The times are only approximations that may be within five minutes of the actual time after fertilization. This error was just human error. It was impossible to take the pictures of the different conditions simultaneously with only one microscope. Another error that occurred in this experiment was technical. About four and a half hours into the experiment, the computer program, Spot Advanced, stopped working and we were unable to take any pictures afterwards. This affected our data because we were only able to collect data from all the conditions for one hour after fertilization. This error reduced the credibility of our data, since our pool of data was small. However, this error did not affect the validity of our data, so we were still able to make conclusions from our results.

If we were to refine this experiment, next time we would probably extend the length of the experiment so that we could observe more cleavages of the embryos. This would allow us to have more data to make conclusions from. We might also make a video of the embryos in each condition starting from the time of fertilization until the last desired cleavage was observed. The video would provide us with extensive data. We would be able to go back and analyze the embryos at any time interval. The movie would also provide more accurate time intervals than we provided using a clock. This would lead to more accurate and valid data. If we were to take a video of the embryos in each condition, we might also want to set up the conditions at 4 different microscopes instead of using one microscope. This refinement would be more time efficient since all the conditions would simultaneously. One last refinement that we would make to this experiment would be to increase the concentration of eggs in the initial egg solution. There were not many embryos to observe on the slides for any condition, so an increase in the egg concentration would lead to an increase in the number of observed embryos in each condition and an increase in the amount of the overall data collected.

We would also suggest for future experiments to be conducted in order to extend our research in new directions. Future experiments might focus on the effects of different concentrations of mercury than the concentrations we chose to study. Other researchers might want to study the effects of much higher concentrations of mercury, similar to the amount of mercury found in polluted bodies of water. They might also want to determine which concentrations of mercury were lethal to sea urchin embryos. Further experiments might study different developmental stages of the sea urchin to observe the long term effects of mercury exposure on the embryos. Other researchers may want to expose the eggs to mercury for a longer or shorter period of time. Additional experiments might expose the sea urchin embryo to mercury at different stages of its development. Other experiments could be done to observe the difference in the effects of mercury exposure of the eggs of different species. These possible experiments would help to broaden the research of mercury exposure, not only of sea urchin embryos, but also of embryos of other animals. The results could be complied to extend the knowledge of the effects of mercury on embryos in new directions.

Back to the Top

V. References

Benoit, Jania. (2004) Interview by Cali McGinn and Ashley Jennings. Mercury. Wheaton College.

Gilbert, Scott F. (2000) Developmental Biology 6th Edition. Massachusettes: Sinauer Associates, Inc.

Herba, E., D. Pojda-Wilczek, AR. Plech, SM. Pojda and R. Szkilnik. (2004) Influence of dopamine on flash visual evoked potentials (FVEP) in prenatally mercury intoxicated rats. Polish Journal of Pharmacology. 56, 415-9.

Jennings, Ashley. (2004) Independent Research Project Collaborator. Wheaton College. Developmental Biology. Professor Morris.

Mercury. (2000) University of Minnesota. October 21, 2004,

http://www1.umn.edu/eoh/hazards/hazardssite/mercury/merchealtheffects.html#Anchor-Toxicity-43793

Morris, Robert, L. (2004) Interview by Cali McGinn and Ashley Jennings. Sea Urchin Development. Wheaton College.

Sea Urchins. (1995) University of California Cooperative Extension. October 25, 2004, http://seaurchin.org/Sea-Grant-Urchins.html#desc

The Health Effects of Mercury. (2004) Oklahoma State University. October, 25 2004, http://www.pp.okstate.edu/ehs/training/mercury.htm

Wilt, Fred H. and Sarah C. Hake. (2004) Principles of Developmental Biology. New York: W.W. Norton & Company.

Wolf-Pollina, Maya. (2004) Interview by Cali McGinn. Image J Computer Program. Wheaton College.

Back to the Top