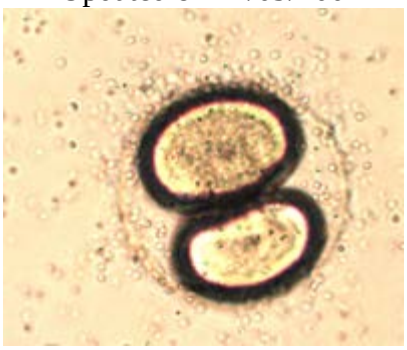
Bio254 Home

Ultraviolet Radiation in the 254nm Wavelength Can Interfere With the First Mitotic Division in Sea Urchins

Matthew Powers

Web Posted on 12/02/2004
Updated on 12/09/2004



Introduction	Materials & Methods	Results	Conclusion	Bibliography

Wheaton College Home

I. Introduction

The process of development in sea urchins is an interesting one because significant conclusions come to in that area can be applied to other arenas of developmental biology. Specifically the process of mitosis and the effects of ultraviolet radiation on it were studied in this lab. The impact of Ultraviolet Radiation is tested only in the knowledge that it is a “bad thing”. This experiment serves as a deeper inquiry into just one of the specific things that Ultraviolet Radiation could impact in an organism. Mitosis as a process is completed at a very regular rate in most organisms. The first mitotic division in the sea urchin species Lytechinus variegatus can be seen one hour after fertilization. With such a specific process happening at a defined time the alteration of a variable, in this case the amount of UV radiation a sample receives, will allow us to see if overall there is a change in the speed, frequency or to some extent success of this mitosis.

Johann Ritter was a German physicist born in December of 1776. He lived and worked in what is now Poland and during his time in the lab, he discovered something quite extraordinary. Using a prism he managed to split a beam of sunlight into is array of colors. He then placed samples of silver chloride at intervals underneath the rays of the spectrum. He allowed the sample to be exposed and after a short while the silver chloride became darker. While doing these experiments he noticed that when he put a dish at the end of the spectrum, near violet, the same darkening occurred despite the lack of visible light. It was not until much later that the magnitude of Ritter’s experiment was realized. He had accidentally discovered Ultra Violet light. (Carleton , 1925) The ultraviolet region of the electromagnetic spectrum is subdivided into three bands termed UVA, UVB and UVC. The subdivisions are arbitrary and differ somewhat depending on the discipline that is interested. Environmental photobiologists normally define the wavelength regions as: UVA, 400-320 nm; UVB, 320-290 nm; and UVC, 290-200 nm. (Jagger, 1967) These wavelengths as a group are known to be harmful to organisms especially when they are not protected.

The process of mitosis that is undergone in organisms like sea urchins is strictly regulated and done in such a way as to provide as little chance for error as possible. In its simplest form mitosis is the equal partitioning of replicated chromosomes into two identical groups with the intent of creating two relatively independent cells. In the majority of marine life this process of mitosis and development takes place not in the protected womb of a mother, but at best is

provided with jelly coating around the outside of the egg. This open development allows us to easily study the development of such creatures, however it also can not protect the egg from things like excessive ultraviolet radiation. (Tevini, 1993) Many eggs of marine animals including sea urchins have a high concentration of mycosporine amino acids that protect them from UV damage. Mycosporine amino acids are obtained through the diet of the adult and are stored in the egg. Of the many things UV radiation can do to the developing organism is cause adjacent thymidines to condense into cyclobutane pyrimidine dimers (CBPDs) which impede DNA replication and transcription. The effects of increased amounts of ultraviolet radiation are likely to be most devastating to organisms that are exposed to sunlight during their developmental stages. Sea Urchins like many organisms have DNA repair enzymes, photolyases. However the damage can't always be fixed. Consequences of these mutations are delayed cell divisions, DNA damage, developmental delays, and abnormalities. UV treatment can cause immediate effects by damaging microtubules and long term effect by damaging RNA and DNA. (Tevini, 1993)
Sea urchins were selected as test subjects because of their spawning reproductive style and therefore easy access to many sperm and eggs. *Lytechinus variegatus* was selected due to the fact that it is gravid in late autumn.

The overall hypotheses for the experiment was that higher than normal doses of ultraviolet Radiation would slow or stop the process of mitosis in *Lytechinus variegatus*. This is significant data to collect and interpret because with the increase in UV light reaching the earths surface, due to human interaction with it, problems may arise with development of marine life and a bank of data may provide helpful in understanding the problem. In this lab we exposed samples of fertilized sea urchin eggs to varying amounts of UV radiation in an attempt to determine the effects on mitosis.

II. Materials and Methods

This experiment began with the process of equipment and specimen collection. To complete our lab we used several different types of tools. The first was a standard Nikon Eclipse E400 spot scope. Observations were done at 10 and 40 times magnification. The other unique piece of equipment required for this lab is an Ultraviolet Lamp. One provided by our biological department specifically produces 254nm radiation. Images were captured through the digital camera associated with the microscope and no magnification was used in relation to the camera. Other materials for this lab include clean slides, pipettes, a timing devise such as a stopwatch, Petri dishes, filtered natural sea water, a ruler, and protective equipment that should be worn during the exposure. Also a room, preferably one that can be closed off with which to do the exposures in should be found. Lastly assorted lab tools and the equipment to cause a sea urchin shedding should be procured.

For this experiment we used the species Lytechinus variegatus to produce gametes. It should be noted that we received two shipments of these urchins, one for each three hour research session that it took to complete the experiment.

The procedure for this experiment was relatively simple however required many reproductions. The preparation for the lab was a significant step because once the trial had begun, it took only a few minutes before work should be done with it. The first step is to find and shed sea urchins using a KCL solution and agitation. This was done for us by either a lab instructor or assistant, however we received a dry, inactivated sample of sperm and a tube with eggs in filtered natural sea water. An important note, Our first control group and first two test groups were completed using the process of removal and squeezing of the gonad to obtain sperm. This process provided less than desirable results and could account for some of the discrepancy in the data for those tests.

After the samples were procured, approximately 2ml of water was placed in a test tube. The end of a dropper was touched to the sperm sample and then mixed with the water. It is important to note that at this point the sample must be irradiated and the mixed with eggs as soon as possible. In our trials we attempted to keep the time under a minute. An equivalent volume of eggs was then suctioned from its respective tube and each sample was placed in its own Petri dish. The dishes were left uncovered so as to not interfere with the incoming radiation. Our exposure room was directly across the hall from the laboratory we were working in and movement back and forth was easy. Once in the room face shields and other protective equipment were put on and the lamp was plugged in. Our lamp was handheld and so getting the lamp at the same height for every trial was paramount. To address this importance literature was consulted about a proper height and with the suggestion of Professor McCafferty a distance of approximately one foot was decided upon. We used a standard ruler to measure one foot off of the desk surface and using the best judgment

possible it was held in place for the exposure. The exposure times for our samples were between 4 and 6 seconds for trials one through four and the last two were around 40 seconds. The ultra violet light lamp, being what it was, produced no visible indication it was on and with the switch being finicky exposure times varied slightly. After exposure the dishes were brought back into the lab and the sperm was pipette into the eggs Petri dish. The solutions were allowed to mix and a cover was placed on the dish. The sample was labeled and put aside. Forty minuets later two to three drops were removed from the dish and placed on a slide with no cover slip. All of the eggs on the slide were counted followed by the ones that had ruptured, entered mitosis, or were just showing fertilization envelope liftoff. This process of observation was done every ten minutes for the next eighty minutes. During the final data analysis a percentage for every trial at every time point was calculated by dividing the number of eggs in mitosis by the total number of eggs in the sample. These percentages were recorded on an Excel spread sheet. Our final data included three controls, four 4 to 6 second exposures and two 40 second exposures.

III. Results

During this lab several important pieces of information were collected. First, based on the data recorded a general trend of decreased speed and success of mitosis occurred when the samples were exposed to UV radiation. Also the two samples that were exposed for longer periods of time showed no real development even after 120 minuets. Data in the graph below depicts the percent of gametes that showed a fertilization envelope and went on to go through their first mitosis. In this lab a collaborator studied the effects of the same amounts of ultraviolet radiation on FELO and for further conclusions on the subject see the paper "UV Exposure to Sea Urchin Gametes and Success of Fertilization Envelope Liftoff" by Amanda Crouch-Smith, 2004.

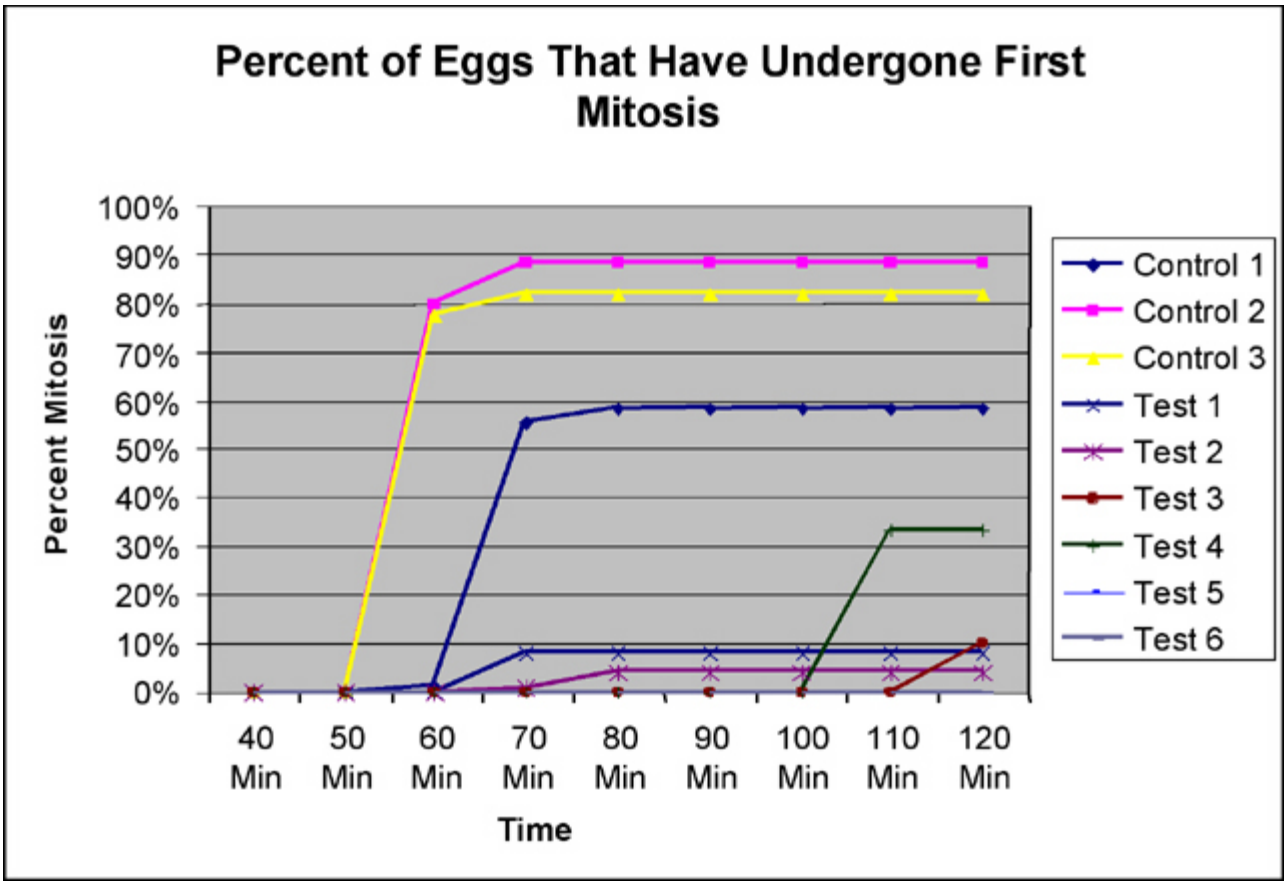


Figure 1.1 This graph shows a trend of decreased speed and success of mitosis in eggs that had been exposed to ultraviolet radiation. Tests one through four were 4 to 6 second exposures and test five and six were 40 second exposures.

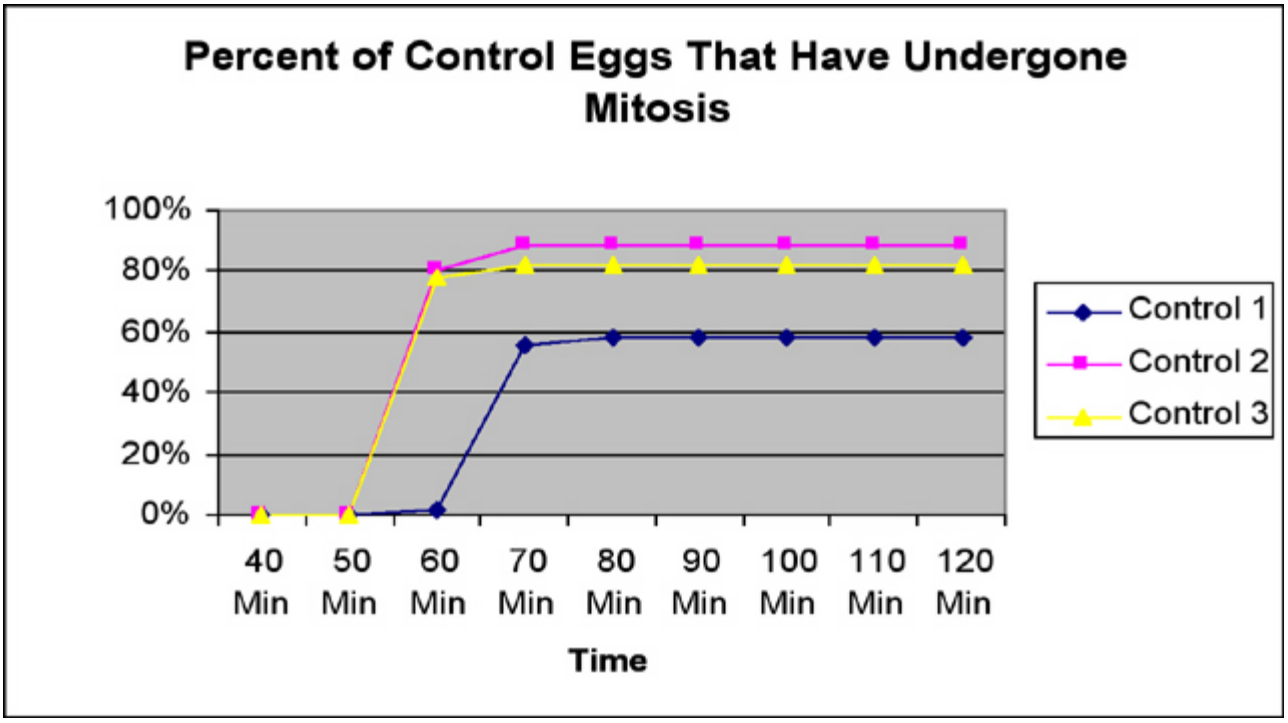


Figure 1.2 This graph shows the percent of eggs in the control group that entered mitosis. As can be seen by the graph control groups two and three had a significantly higher fertilization success rate than control one. This decrease could be attributed to the fact that it was the first trial done and the kinks were being worked out, and because the method of sperm collection was done through removal of the gonad and not a KCL shedding. The sperm was observed under a microscope after attainment and the sample from control group one looked less healthy and mobile than the samples procured for controls two and three. This could have suggested the overall health of the sperm and therefore their ability to fertilize eggs.

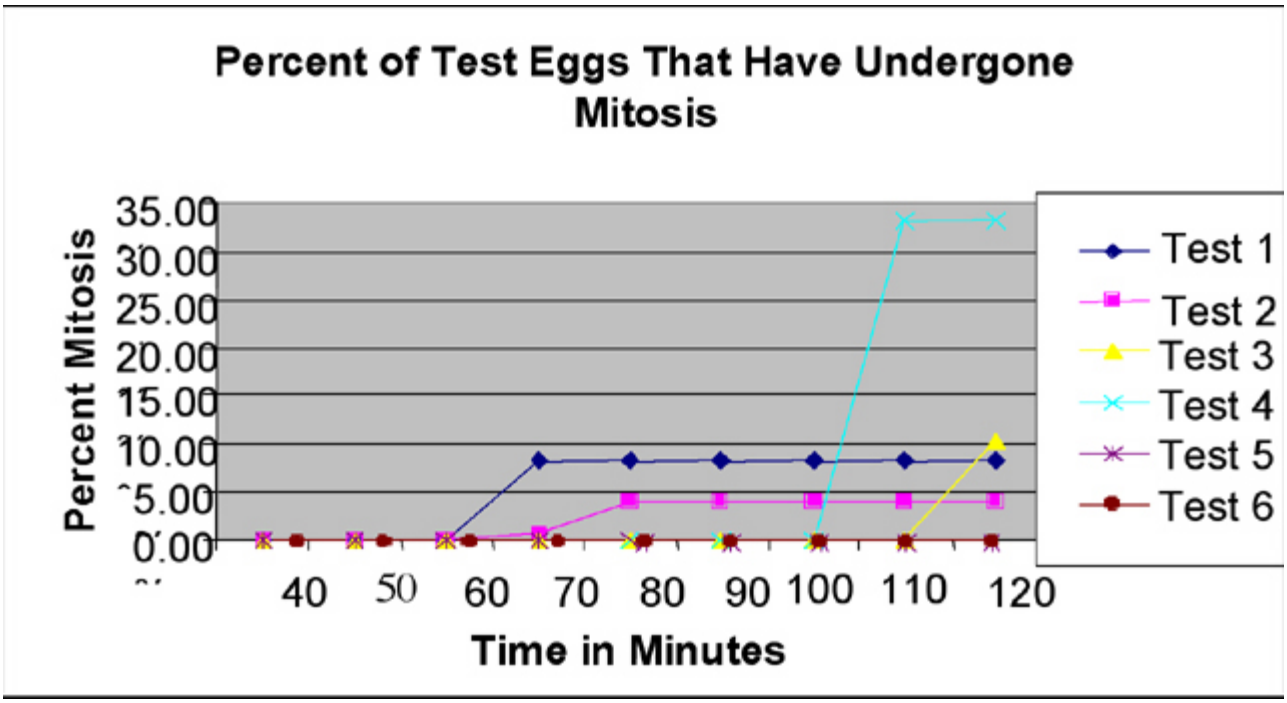


Figure 1.3 This graph shows the percent of eggs in the six test groups that showed some mitosis after exposure. Test groups one and two were done on the first day of the experiment. Tests three through six were done a week later on the second day of experimentation.

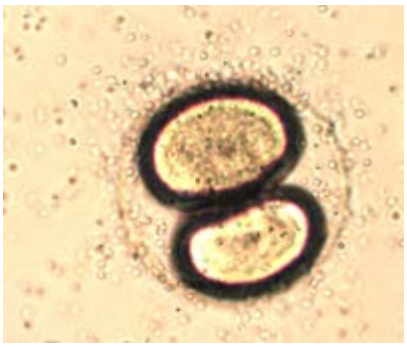


Figure 1.4 This image was taken with the E400 Nikon Scope using the 10x objective and illustrates mitosis in a sea urchin egg.

The general tend of data seems to suggest that even when samples are exposed to minimal amounts of ultraviolet radiation there is a significant decrease in percent of successful mitosis. With the exception of one trial the percent mitoses were down around 10% instead of 80% and happened on average 20 minuets later.

IV. Discussion and Conclusions

Of all the results collected the data seems to support the original hypothesis. Mitosis occurs more slowly and less successfully when the sample is exposed to ultraviolet radiation. In the test groups studied the samples that were exposed for four to six seconds showed a drastically reduced mitotic success rate while also being delayed for an average of twenty minutes. Test groups labeled five and six were exposed for forty seconds and showed no change even after 120 minuets. Further experiments could include forty second exposures that are observed for a longer period of time to discern if the fertilization is still viable. Intermediate exposure times could also be tested.

Sources of error in this lab are numerous and can even be seen in the data that was collected. The shedding styles used in this lab were different and can be seen in Figure 1.1. The success of mitosis is drastically reduced in control one and could be attributed to the fact that a different shedding style was used. This being the case tests one and two represent data that may or may not be commensurate with the rest because they too used the day one sperm. Other sources of error include the height of the UV light not being consistent through the tests despite the best effort to hold it in place. This could be a needed refinement if this lab were to be repeated. Some form or structure could be constructed to support the light at an exact height. Error could also come from the time between the exposure and the mixing of the Petri dishes being too long. A combination of or none of these things could present problems in the data of this lab, but overall the gathered information seems to be relatively consistent.

This lab serves to show that Ultraviolet Radiation harms the development of sea urchins. This experiment was conducted in a lab and therefore can only imply the fate of marine organisms if the amount of UV radiation reaching them increases. Field tests and studies would need to be done to further explore this topic for information that can support real world happenings.

V. Bibliography

Lab partner and collaborator Amanda Crouch-Smith Author of:

"UV Exposure to Sea Urchin Gametes and Success of Fertilization Envelope Liftoff"

Carleton, E. *The chemical action of ultraviolet rays*. New York, The Chemical catalog company, inc., 1925

Jagger L. *Introduction to Research in Ultraviolet Photobiology*. Prentice-Hall. New Jersey, 1967.

Moseley, H. *Ultraviolet and laser radiation safety*. Department of Clinical Physics and Bioengineering, West Scotland Health Boards, Glasgow G12 8SQ, UK. 1994.

National Research Council (U.S.). Committee on Chemistry and Physics of Ozone Depletion. *Causes and effects of stratospheric ozone reduction*. Washington, D.C. National Academy Press, 1982

Tevini, M. *UV Radiation and Ozone Depletion: Effects on humans, animals, plants, microorganisms and materials.* Lewis Pub. Boca Raton, 1993.