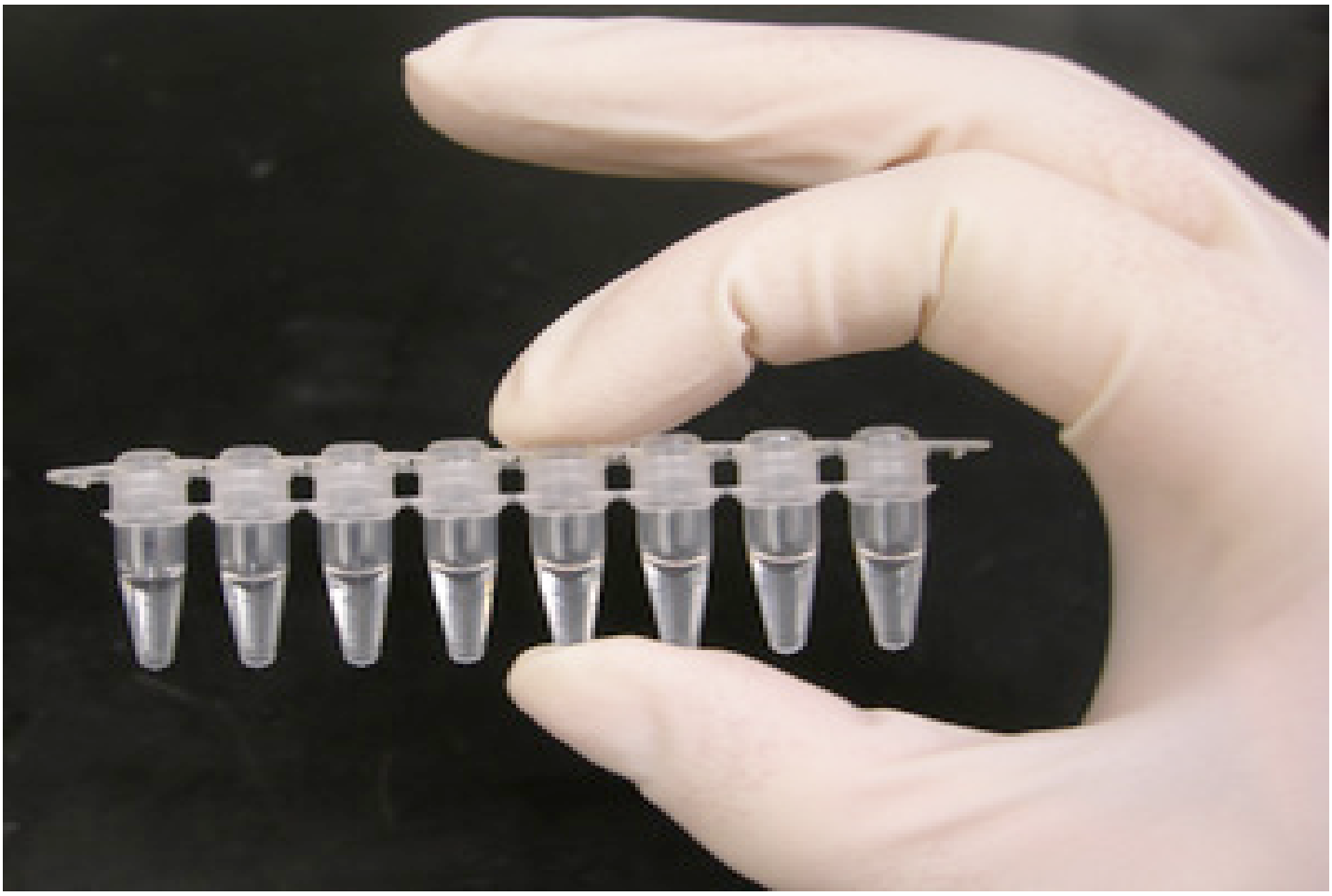
The Pingry Community Research (PCR) Journal



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DNA Barcode of *Anoplophora glabripennis*

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ABSTRACT

DNA barcoding, a relatively modern taxonomic method of identifying species, is the process of isolating the cytochrome c oxidase 1 gene (CO1) in a species in order to correctly classify it (11).The CO1 gene is unique in every species. For our project we discovered the cytochrome oxidase subunit I of two different varieties of the Asian long-horned beetle (ALB), one from New York and one from Chicago. We designed unique primers to isolate the gene, as it is different from the typical larger CO1 region isolated for a barcode. We wanted to correctly find the CO1 subunit barcode and see if this subunit of the CO1 would correctly identify the ALB, just like the larger CO1 region.

INTRODUCTION

Taxonomy is the study of classifying organisms (10). Today, this field is very important in observing evolution in species and the environmental effects on species. In the past, scientists have used speculation to identify organisms based on differences such as color, shape, and habits (10). This old method is ineffective in classifying species which have identical appearances, but different genetics.

Barcoding helps taxonomists because an organism can be classified by the genome of the species, and not solely based on the organism's appearance. The barcode can be found in all stages of the life cycle, even if the sample is damaged (11). Another benefit of DNA barcoding is that non-experts can easily identify species using a straight-forward procedure (11). The Asian long-horned beetle (ALB), scientifically known as *Anoplophora glabripennis*, originated in southeastern Asia (3). It was first spotted in America in 1996 in New York and has been spreading to other regions of the country ever since (3). The ALB is extremely harmful to the environment because it feeds on various species of trees. It is considered one of the most invasive nonnative species in the nation. As a result, forest and quarantine programs have spent millions of dollars attempting to eradicate the ALB and restore damaged plant life (3). The beetle's appearance, which is black with white spots and approximately one inch in length, makes it seem harmless enough. Detection of the ALB is very difficult since recognition is only possible during maturity periods in the spring and summer(4).

MATERIALS AND METHODS

In order to find the DNA barcode of the ALB, we ran two separate procedures during the duration of this project. Before running the experiments, we first had to obtain beetles, design primers, and find DNA Isolation and Polymerase Chain Reaction (PCR) Protocols. We were given two beetles by the Forest Quarantine Facility in Ansonia, Connecticut. One originated from a New York infestation and was labeled as part of the 5SNB strain. The other originated from the Chicago infestation and was included in the 17UIC strain.

We designed our own primers to isolate the CO1 subunit gene in the ALB (1). In the end we created four unique primers, two for the initial PCR and two for sequencing. Of the two PCR primers, one was forward and one was reverse. For the sequencing primers, both anneal forward. We designed primers with a high GC content using the *Anoplophora glabripennis* mitochondrial genome on NCBI (2).
The primers we designed were:

PCR Primers:
Forward: Dmax.ALB.PCRF1:
5' GGTGCATGAGCTGGAATACTTGG 3'
Reverse: Dmax.ALB.PCRR1:
5' CCTAAAGTCCCAAAGGCTTC 3'
Sequence Primers
Dmax.Alb.Seq.F1:
5'GTTCCATTAATACTAGGTGCCCCAG 3'
Dmax.Alb.Seq.F2:
5'CAACTGTAATAAATATACGACCCC 3'

We ordered our primers from IDTDNA.com. The primers were sent to us in lyophilized form, meaning without water. We diluted two stocks of primers: a personal stock which was a 100 μM solu-

tion, and a working stock which was a 10 µM solution (8).

Before finding the barcode for the Asian long-horned beetle, we practiced the procedure on a stink bug (*Halyomorpha halys*). To isolate the DNA, we used the DNeasy Blood and Tissue Kit from Qiagen and followed the procedure in the kit (6). After we obtained the DNA, we ran the PCR Protocol for Taq 2X Master Mix (7). Two specific parts of the experiment that were unique to our project were the annealing temperature and time of extension. Our annealing temperature at 48.3 °C and the extension time was 45 seconds. We used these procedures for both the stink bug practice trial and the Asian long-horned beetle trial.

To test if the isolation and PCR was successful in both trials, we used gel electrophoresis to see if our DNA was amplified. We used a 1% 50 mL Agarose and Buffer TAE Gel. For both the practice test and actual procedure, we ran two GMO-control PCR products lanes, insect PCR product, our primers, and a ladder. After running the electrophoresis, we photographed the gel to see if the DNA in the lanes were present. We mixed 7.5 µl of PCR DNA along with 7.5 µl of dH20 and 3 µl of DNA-staining dye in each of our insect and control lanes. For the primer lane, we combined 1 µl forward PCR primer, 1 µl reverse PCR primer, and 1 µl DNA staining dye. For the ladder lane we used 3 µl of of Tri-dye ladder.

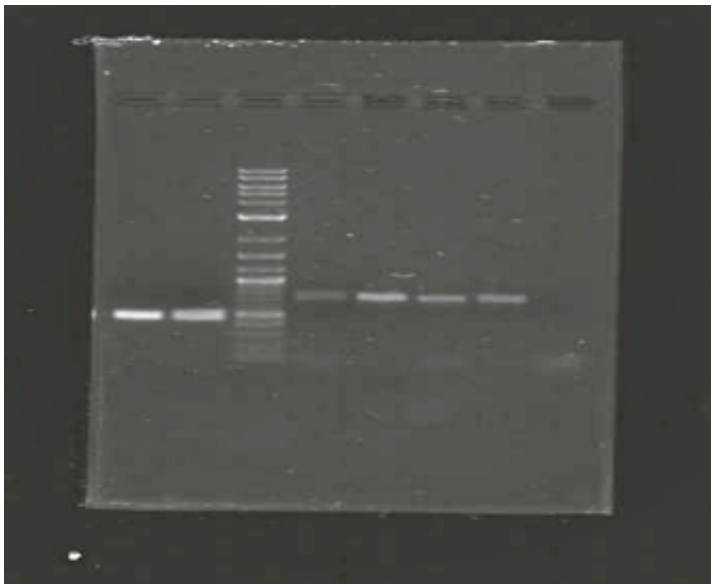
Our last step in this project was sending our PCR DNA for sequencing. We used the company Genewiz, where we sent "pre-mixed" samples for the identification of our sequence. First, we purified our PCR DNA using the affymetrix purification protocol (9). Then, we followed Genewiz's pre-mixed guidelines to dilute our purified DNA with the appropriate amount of water and primers (5). After sending our DNA, we received the sequence online from Genewiz.

Finally, we used the Barcode of Life Database (online), which houses many barcodes, to confirm that our shorter sequence was a viable alternative to the longer CO1 region and to confirm that we found the correct barcode for the ALB.

RESULTS

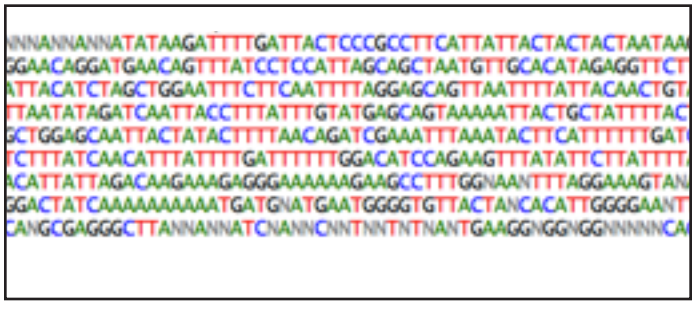
Our gel electrophoresis showed bands proving that our DNA isolation and PCR were successful. Our control lanes, ALB DNA, and ladder all appeared. Against the ladder, our ALB DNA is near the 720 BP marker, roughly the size of our extracted and cut DNA. The labeled image below is the photograph of our gel.

In addition, after successfully running the gel, we sent PCR product and received our sequence results. From the eight samples we sent, we received data (sequences) for each.

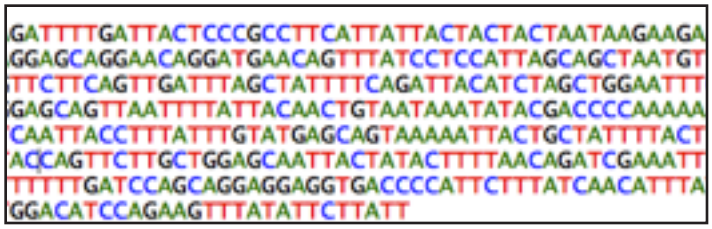


Gel electrophoresis, proof of DNA present (allowed us to move forward)

v5SNB Sequence



17UIC



Our shortened, final barcode (COI subunit)

DISCUSSION

We successfully completed our original goal, which was to obtain the CO1 subunit and test it as a successful barcode. We checked our result with the traditional and more commonly known barcode. Our shorter sequence matched with 100% accuracy. In the future, if other species could be barcoded in the same shorter fashion, the taxonomic process would be shorter, more cost-efficient, and easier for non-experts to do.

Additionally, we set out to find out what skill level was required to test a known barcode. We concluded that with cursory biology knowledge (knowing how to run a PCR and having access to some biotechnologies), one can perform this procedure with relative ease.

In the case of an outbreak of the ALB or other invasive species, a barcode allows quick and correct identification. Barcoding is a modern and effective taxonomic method. In the future, scientists hope to barcode all species.

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Determining the Light Intensity and Type of Light on Respiration of Algae

By Nicole Witte'13, Ashley Zhou'13, Ryan Toomey'13, and Adam Palmer'13

ABSTRACT

Algae are very important plants to the earth. Algae can be grown and used as anything from agar to an energy source. To best grow algae, we must find under what conditions algae thrives. To do so, we grew algae under multiple different light intensities and two different types of light (white and ultraviolet). By measuring the amount of dissolved oxygen with a lambda sensor, we were able to determine which tubes were doing the most photosynthesis (or respiration). Our results demonstrate that white light seems to be most effective for growth, but whether light intensity is at 100% or 4%, a viable amount of oxygen is still produced.

INTRODUCTION

Algae, of which there are thousands of species worldwide and which grow in unusual conditions like snow, ice, and New Jersey winters, have been found to have many uses. Besides being an important food source, algae can also be used in stabilizing substances, agar, fertilizers, as pollution control, and as an energy source (1). Algae are a renewable organic carbon source and, additionally, since such a large part of the earth is covered by water, and algae grow in water, the mass growing of algae would not only not avoid the use of land, it could truly be a potential replacement for fossil fuels. Furthermore, because algae have the ability to fix CO_2, and fix it more efficiently than terrestrial plants, it has been proposed that they might be able to remove some of the CO_2 from the atmosphere (2).

In order to grow in large quantities, it is necessary to determine under what conditions algae grow, as measured by the amount of O_2 released from the best algae sample. Although there are many factors we could test, such as temperature or humidity, we will be testing under which light algae grows best: white light, UV light, or no light. We will collect algae samples from the Pingry pond by the tennis courts and subject them to the three different kinds of light, then measure the amount of oxygen emitted. We hypothesize that the samples receiving no light will produce the least oxygen, the samples receiving UV light will produce the most oxygen, and the samples receiving white light will produce an amount of oxygen less than that of the UV samples but more than that of the no light samples.

MATERIALS AND METHODS

We conducted an experiment on the rate of photosynthesis of algae when exposed to increasing light intensities using an instrument that reads oxygen production (a lambda sensor). We used 0% light, 20% white light, 40% white light, 60% white light, 80% white light, 100% white light, and lastly UV light.

To grow the algae to produce the amount of oxygen we would need to test, we removed algae from an ample source from the tank in Room 106. We then filled a container up with 1 gallon of water, and poured 15 mL of miracleGRO fertilizer into the container. We then poured the contents of the container into four flatbeds and added 100 mL of algae water into each. We grew our continuous culture of algae in those containers for five days. They were placed under a light source, and the combination of light and fertilizer helped the algae grow. Once we had enough algae to sample from, we were able to use them to separate into multiple tubes for experimentation.

We used 3 culture tubes per light source, filling each 50 mL tube to the top. There were 21 culture tubes of algae and 3 tubes for each light intensity. We covered each tube with pieces of film so the correct amount of light would be able to penetrate through to the different algae samples. 3 tubes were covered in tin foil to ensure no light went through, 3 were left uncovered, 3 were wrapped with 1 film each, 3 were wrapped with 2 films each, 3 were wrapped with 4 films each, and the last 3 were placed in a UV light box that emitted fluorescent UV light. In order to determine the amount of light emitted through to each tube, we used Image J,

an image processing and analyzing program. We placed 1 film, 2 layered films, 3 layered films, and 4 layered films inside a photocopier to get a digital grayscale image to run through the Image J program. Using the digitalized picture and the photo-analyzing program we calculated the amount of light emitted through the films so we knew how much light each tube was receiving. In order to conserve light energy and hinder possible error, we placed all white light tubes under the same light source in the flat bed lights in Room 108 and 3 under a UV light source.

Each test tube was measured at the same time in order to ensure consistent results and that the rate of photosynthesis (and cellular respiration for the 3 tubes in complete darkness) producing our oxygen would have the same time increments between measurements. Oxygen saturation was measured to the nearest tenth of a percent by the lambda sensor, as well as milligrams of oxygen dissolved per liter. All data was taken down in lab notebooks and then organized into graphs to represent our results.

RESULTS

Our results showed that the tubes with no film, exposed to 100% white light, overall produced the most dissolved oxygen over time. The tubes covered with aluminum foil, exposed to 0% white light, dissolved the least amount of oxygen over time (Figure A). The tubes exposed to 59% white light produced more dissolved oxygen in the first 7 days than those exposed to 100% white light but the numbers gradually declined over time below the tubes exposed to 100% white light. The tubes exposed to 100% UV light overall produced less dissolved oxygen than all of the algae tubes exposed to some kind of white light (4%-100%) (Figure A). By day 21, the tubes covered in 2, 3 and 4 mesh films (exposed to 32% white light, 13% white light, and 4% white light) all produced the most dissolved oxygen for that particular reading. However, over the course of 21 days, the results showed that there was a decline in dissolved oxygen. This can be correlated to the decrease in light intensity except for the outlier of UV light. The tubes exposed to 100% UV light, although their light intensity was at 100%, still had on average less dissolved oxygen than that of all the tubes exposed to any percentage of white light above 0%. Throughout the 21 days, all tubes containing algae consistently produced less and less dissolved oxygen as the days continued.

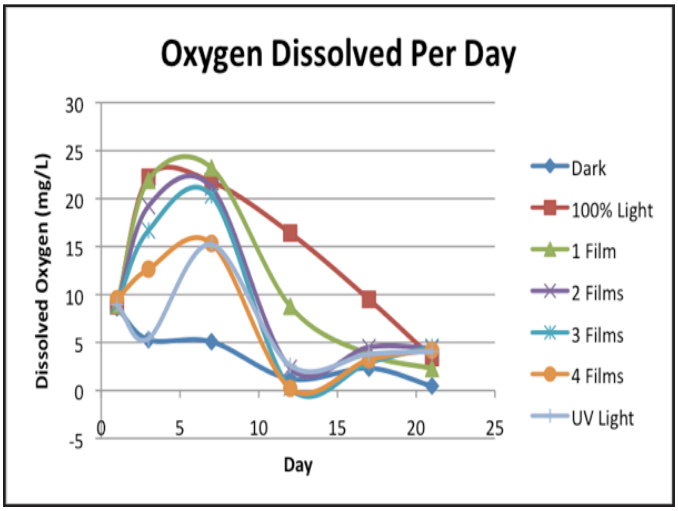

Figure A: Dissolved Oxygen per Day
Graph of averages of dissolved oxygen (mg/L) per different light intensities on algae tubes

DISCUSSION

"No film" had the most mg of oxygen dissolved per liter, even more than UV light. We expected this result because these tubes should have been performing the most photosynthesis because they would receive the most light energy. As pieces of film were added, for the most part, there were fewer mg of dissolved oxygen per liter. The mg of dissolved oxygen produced by the algae under UV light was less than that in all tubes exposed to any percentage of white light; however, UV light still had more dissolved oxygen than no light at all. Each sample started with approximately 8.99 mg/L of dissolved oxygen. The tubes with 100% white light produced the most oxygen of all the tubes whenever measured (up until the last reading). These tubes were performing the most photosynthesis, and therefore contained the most dissolved oxygen, but eventually dropped below that of the tubes with 2 pieces of film, 3 pieces of film, 4 pieces of film (32.65%, 13.11%, 4.01% of white light, respectively) and UV light on the last day which we believe to be due to human error or possible algae deterioration. Throughout the experiment, the mg/L of dissolved oxygen decreased under all intensities of light. This means less and less oxygen was being produced from photosynthesis. This is possibly due to the fact that small organisms inside the algae were eating and deteriorating the algae, leading to less photosynthesis occurring, causing the numbers to decrease. The amount of dissolved oxygen dropped for tubes exposed to light intensi-

ties of 32%-4% white light and 100% UV consistently but somewhere between day 12 and day 17 the amount of dissolved oxygen started to increase. An explanation for this could be the organisms eating the algae had died and were no longer interfering with the process of photosynthesis. A similar process may have occurred in the tin foil wrapped tubes (receiving 0% of light) because they also were producing oxygen, but through the process of respiration because they had no light to perform the process of photosynthesis. In tubes exposed to 100% - 59% white light, the dissolved oxygen decreased throughout the 21 days. This is possibly because whatever was interfering with the algae (possible organisms or dying leaves) did not die/continued to interfere, causing a consistent decrease in dissolved oxygen .

Approximately 75% of the oxygen of Earth's atmosphere is the by-product of photosynthetic algae and cyanobacteria (3). Its many resources and uses make algae very important in many aspects of our lives. We have concluded from our experiments that algae grows best in 100% light, but still produces a viable amount of oxygen when only exposed to 4.01% all the way to 100% and UV light. Our results found that 100% white light and 4% white on day 7 only differed 6.4 mg/L of dissolved oxygen. The less light they receive, the less photosynthesis occurs, but it still occurs. If we can figure out how to maximize the growth of algae with further experiments with light intensity and growth conditions, we will be able to harness it and maximize all possible uses with the least light energy required, leading to a better environment.

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Biophotovoltaics: Generating Electricity from Moss

By Jennifer Guo (V)

ABSTRACT

The search for renewable energy sources has occupied much of research for the past few years. One of these new energy sources currently being tested is Biophotovoltaics, which utilizes the process of photosynthesis in plants to generate electricity. In my project, I attempted to replicate the moss table, which is a concept product using Biophotovoltaics. The goal of my project was to discover whether photosynthesizing moss is actually able to generate electricity. The results demonstrate that moss is able to generate electricity, and factors which affect the amount of electricity generated are the amount of water and amount/type of lighting.

INTRODUCTION

Biophotovoltaic devices (BPVs) are a new form of sustainable energy, currently undergoing research. Sustainable energy research is very important at the moment because the availability of fossil fuels is being quickly depleted. Biophotovoltaics utilizes the photosynthesis of living organisms to generate electricity. This project is modeled after the concept of moss tables, and utilizes the following process: when moss undergoes photosynthesis, it releases organic compounds into the soil, like carbohydrates, proteins, and lipids. The bacteria in the soil break down these organic compounds, and give off electrons as a by-product. Electrodes in the moss pot then capture the electrons and put them to use (1). This research regarding Biophotovoltaics is important because it offers the possibility of a novel sustainable energy source. If it is possible to generate electricity from moss, then it is also likely that Biophotovoltaics can be done with other photosynthetic organisms. Grass and algae are two ex-

amples of other photosynthetic organisms that have been studied and found to be able to generate electricity (2,3). Biophotovoltaic devices are able to harness energy that would otherwise be wasted, and are also much cheaper and easier to maintain than solar panels (although they cannot generate as much electricity) (4). The main goal of this project was to find out whether or not moss could be used to generate electricity, and if so, how that energy could be more efficiently produced. According to my hypothesis, pots with moss should generate more electricity than pots without moss (the control). Another, more specific, hypothesis tested was that when both the moss pots and controls were watered to saturation, the moss pots would start out with a higher amperage than the controls, but would experience a faster decrease in amperage, due to the difference in water retention capabilities.

MATERIALS AND METHODS

In order to test the amount of electricity generated by the moss pots, a system was set up with four controls and four pots with moss. The pot setups consisted of plastic cartons, with dimensions of roughly 10 cm x 10 cm x 9 cm (length x width x height), and 9 holes in the bottom of each. A small piece of a plastic bag was inserted into the bottom of these plastic cartons to prevent leakage of water from the holes in the bottom. In both the controls and moss pots, "Scott's seeding soil" was used for soil. Two electrodes were also included in the setup, which were inserted through slits cut on one side of each plastic carton. The copper electrode was placed through the top slit, while the aluminum electrode was placed through the bottom slit. The copper electrode was approximately 3 cm from the top, the aluminum electrode was approximately 3 cm from the bottom, and there was approximately 3 cm of space in between the two. The copper electrode was placed on top to better attract the electrons from the decomposition of organic material by bacteria. Copper and aluminum were the two materials chosen because they have different electric potentials. Copper equilibrium has an electrode potential of +0.34 V, while aluminum equilibrium has an electrode potential of -1.66 V (5). This means that the copper ions are more easily reduced than aluminum ions (or will more easily accept the electrons) (6). This also allows copper to attract the majority of the electrons created through the photosynthesis of moss. The controls did not have anything additional added to these materials. The moss pots had a single layer of moss added to the top of the soil, which was pressed down in order to become more integrated into the soil.

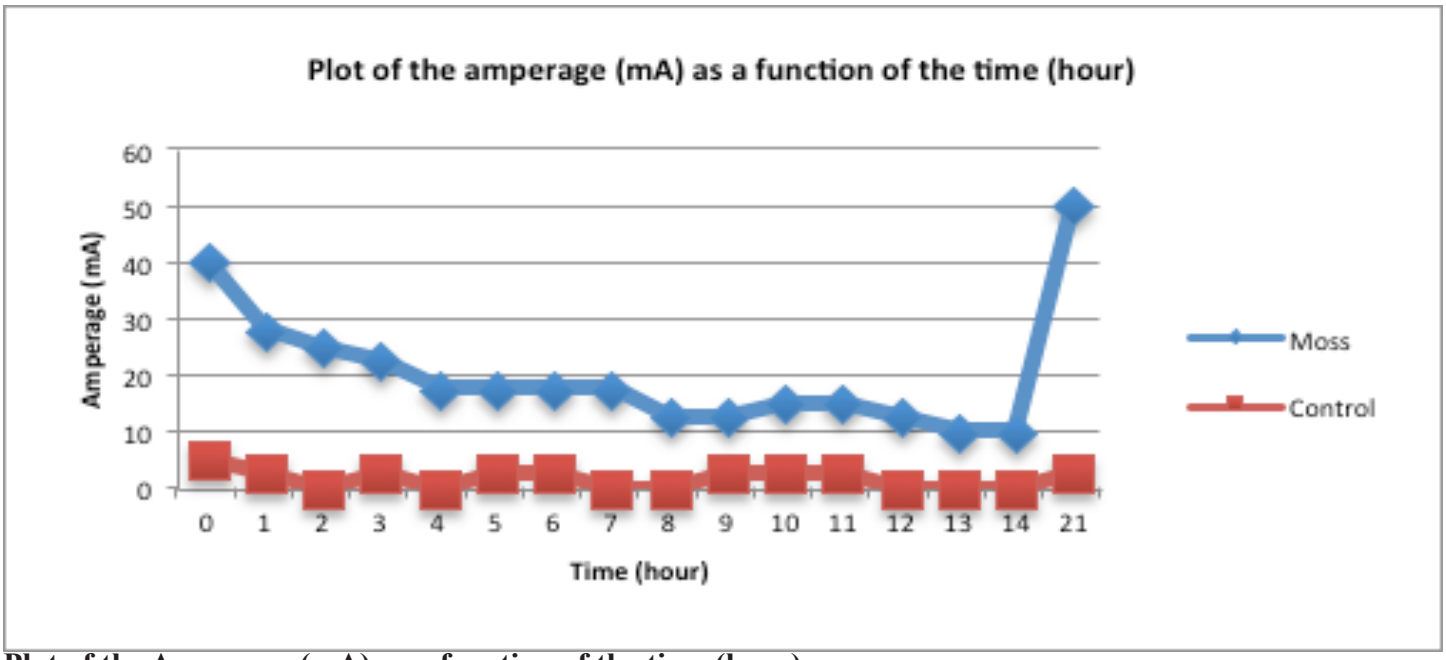
The methods used to test whether or not the moss pots were able to generate electricity were as follows. Before watering either the controls or moss pots, both a voltmeter and ammeter were used to measure the voltage and amperage of each pot. Voltage measures the pressure or force of electricity, whereas amperage measures the amount of electricity used (7). The voltage was basically the same for every single pot, whether control or moss, which was consistent with expectations, because the voltage depends only on the electric potential between the two electrodes (constant since the same electrodes were used for all pots). The amperage was the determining factor of the experiment, because it measured the amount of electricity that was actually being created. After these two measurements were taken, all eight pots were watered to saturation point. The voltage and amperage were both recorded again. Then, no more water was added, and the measurements were re-taken every hour afterwards.

RESULTS

The results were obtained by repeating the same procedure daily over the process of two weeks.

time after watering (hours)
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
21

time (hours)	light status
1pm	natural light + house light
2pm	natural light + house light
3pm	natural light + house light
4pm	natural light + house light
5pm	natural light + house light
6pm	natural light + house light
7pm	natural light + house light
8pm (dark)	natural light + house light
9pm	house light
10pm	house light
11pm	house light
12am	house light
1am	house light
2am	no light
3am	no light
11am	natural light + house light



Plot of the Amperage (mA) as a function of the time (hour)

The procedure began every day at 1:00 p.m., and ended at 11:00 a.m. the following day. At 1:00 p.m. every day, all pots would be watered once to their fullest capacity (where there was water dripping out of the bottom of each pot). This was done to ensure that there was an identical amount of water in each pot, to eliminate one of the unknown factors. Then, a measure of the voltage and amperage was taken immediately after watering, and once every hour afterwards until 3 a.m. in the morning. Measurements would then be taken once again at 11 a.m. the next morning, and then the cycle would start over again at 1 p.m., two hours later. The status of the light at the time the measurements were taken was also noted, as the set-up was placed near a large screen window, so light was a factor.

The data table on the previous page is an average of all of the measurements recorded over the two-week span. This table includes only the measurements of amperage and not voltage, since voltage was fairly constant among all pots at all times (~.66 V), whether they contained moss or not. The left most column contains the time elapsed after the pots have been watered, and corresponds to the 2nd column from the left, the time of day. In the 3rd and last column is the status of the lighting and states whether there is house lighting, natural lighting, both, or neither.

The graph above is obtained from the results in the data table. It shows the plot of the amperage in milliamps as a function of the time elapsed in hours. The line marked by diamonds represents the averages of all values recorded over the two week span for the four moss pots, and the line marked by squares represents the four controls. No trendline was added, since it is unsure what type of trendline the data should follow. However, it is apparent that as the amount of light decreases, so does the amperage, as is shown by the line connecting the points.

DISCUSSION

As shown by the data and graph, there is a significant difference in the amperage between the moss pots and the control. That means that the moss does make a difference, though the data doesn't describe exactly how. However, the voltage was constant along all pots, so according to Ohm's Law (V=IR), there must be a difference in the resistance to result in a difference in the amperage between the moss pots and the controls. Because the amperage of the moss pots is higher than that of the controls, it must mean that the resistance of the moss pots is lower than that of the controls. That should indicate that the resistance of the soil affects the generation of current.

Though there were no significant differences in amperage between the controls themselves (only varying between 0 and 5 mA for the large part), there were between the moss pots. As per the data, it seems that either the amount of water in the soil (directly related to the time elapsed), the amount of light hitting the moss, or both, factor into the current generated. Unfortunately, the two factors were not isolated and tested separately, which is something that will be talked about in future research. So it is

possible that the amount of water in the soil affected the electricity generated, because it definitely would have changed the resistance of the soil (more water retained means less resistance). It is also possible that the amount of light, or type of light, affected the electricity generated, as it affects the rate of photosynthesis of the moss. This is demonstrated in the data table, where there is a significant difference in the amperage between natural lighting/indoor lighting and no lighting at all.

It is likely that both water and light are factors, as can be seen in the graph. The amount of water most likely accounts for the small differences within different recordings of the same type/strength of lighting, while the large differences in amperage can be accounted for by the type/strength of lighting. As can be seen in the graph, there are large differences in amperage between different types of lighting, and small differences between same types of lighting, but different amounts of water (shown as time elapsed). This is most obvious when looking at times when the sun is the strongest (around noon). At the points when the sun (natural lighting) is the strongest, the amperage recorded is much higher than all other recordings. At the points where there is no light at all, the amperage recorded is the lowest of all recordings. However, there are also small differences within periods of time where the status of the lighting has not changed. This is most likely due to the decrease in amount of water; however, it could also be due to error (though unlikely due to the amount of trials undergone) or small uncontrolled changes in natural lighting.

There are a lot of aspects of this project that could be improved upon in the future. Firstly, to address the problems inherent in the project itself, the effects of the amount of water and the effects of the amount/type of lighting should be isolated. Though there can be inferences drawn from doing the project without separating the effects of the two, the conclusions will be much more accurate if the effects of the two separately are known. It would eliminate much of the error inherent in the calculations (such as the inability to determine the actual amount of light, and so having to categorize the status of the lighting). Future research could also be done to examine how the type of photosynthetic organism used affects the electricity generated, as well as the type of soil (or type of bacteria within the soil). In doing this future research, something we will hopefully find out is how exactly the photosynthetic organisms affect the electricity generated, and how the results of the process can best be maximized.

ACKNOWLEDGEMENTS

I would like to thank my Biology teacher, Mr. Maxwell, my Physics teacher, Mr. Jenkins, my parents, and my uncle for supporting my efforts in this project, and for making it an extremely worthwhile experience.

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Comparing Skin Pigmentation and Melanin Production to the Mutagenic Effects of Ultraviolet Rays Using *Sordaria fimicola*

By Ellen Cahill'13

ABSTRACT

UV light exposure activates melanocytes to produce the pigment melanin, which colors skin as it moves to the surface. Humans with darker skin have more melanin and it has been speculated that they may have a biological advantage in the form of resistance to UV damage and skin cancer. Therefore, the theory of photoprotection from melanin levels was investigated by using the fungus, *Sordaria fimicola*. The spores of two types of *Sordaria fimicola* were grown and exposed in varying concentrations and times to UV light, simulating skin pigments. Wild-type sordaria grew more often than did tan type after exposure. These results explain and help support the claim that those with darker skin do not get skin cancer as often as those with lighter skin, by demonstrating that darker skin pigmentation imparts a biological advantage against DNA damage from UV radiation.

INTRODUCTION

The connection between skin pigmentation, melanin production and UV light radiation is directly related to skin carcinogenesis (2). UV light exposure activates melanin production in the melanocytes, and skin cells hit by radiation become colored as they move to the surface. In other words, the more melanin you have in your skin, the darker your skin color should be. The epidermal pigment, melanin, which absorbs ultraviolet rays and limits the penetration into skin tissues, supposedly prevents the incidence of "ultraviolet-induced skin cancer by shielding cell nuclei and reducing DNA damage formation" (1). Yet, many scientists have not been able to produce conclusive results. What is known is that dark skin offers more protection from UV-induced DNA damage than light skin because of the higher melanin levels, but what is not known is whether or not there is a photoprotective advantage from facultative pigmentation (2). Studies by FASEB Journal have shown that amount of pigmentation affects the probability of the presence of skin cancer, which most likely indicates the photoprotective benefit of melanin (3). For example, the rate that Caucasian people get skin cancer is 50 times higher than that of African Americans (3). What remains to be known is whether or not the apparent photoprotection is due to melanin alone as a "UV filter" or if there are other properties involved (3). Although much is known about melanin in relation to UV light, my experiment will try to determine whether or not skin pigmentation and the existence of melanin reduces the risk of skin cancer, therefore imparting a biological advantage (2). The goal of my research is to compare skin pigmentation and melanin production to the effects of ultraviolet rays using the fungus Sordaria Fimicola in two forms. My hypothesis is that the higher production rates of melanin in darker skin tones protect an organism from much of the UV damage, making tanning beneficial for those who produce more melanin. Yet, there is a point at which melanin

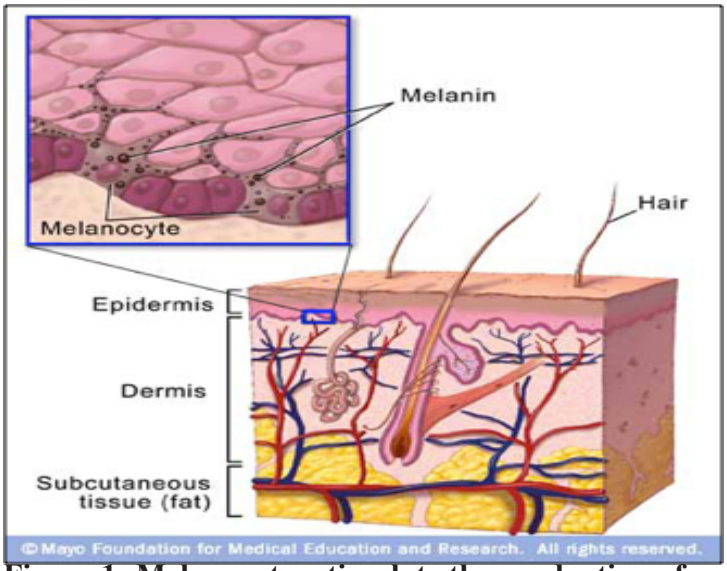


Figure 1: Melanocytes stimulate the production of melanin in the epidermis layer of the skin.

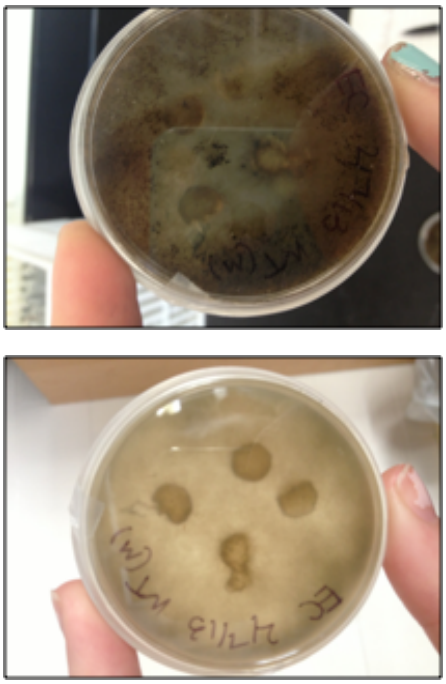


Figure 2: Tan and wild type Sordaria fimicola mating agar

will not protect the skin once a certain amount of UV radiation is reached. All in all, I am attempting to relate melanin, skin pigmentation and cancer-causing UV light damage.

MATERIALS AND METHODS:

Part 1: Growing/Mating Spores

In order to compare skin pigmentation and melanin production to the effects of UV rays, I used the fungus sordaria fimicola in two types: tan and wild. First, I used growth agar to grow spores from the fungus on plates and incubated them for one week at room temperature. To collect the spores, I took plugs out of each growth agar plate to be used in sordaria-mating agar plates, made with 3.41 grams of mating agar and 100mL of water. I used a sterilized plug tool and transferred the plugs from the growth agar onto mating agar plates using a loop, proceeding to incubate these for one week at room temperature.

Part 2: Finding Concentration/Dilution Series

Next, I set up six 15 mL test tubes each with 9 mL of distilled water. In one of these tubes, I poured the water onto one of the tan-type mating agar plates, scraped the plate to loosen the spores, and poured the water and spore mixture back into the test tube. I repeated this for the wild-type. To find the concentration of the spores in the two tubes, I used a corpuscle-counting chamber to

Figure 3. Removing plugs from growth agar with a loop.

count the spores from four squares under a microscope and averaged the number. Since the number of spores is calculated in cubic millimeters, I converted this into cubic centimeters so the average spore count per box multiplied with 104 is equal to the concentration. This came to be 2.5 x 105 for the tan-type and 6 x 10^4 for the wild-type. Next, I set up a dilution series with the remaining test tubes (3 tubes per fungus type). I poured one milliliter of the original tan-type tube into the second tube that had 9 mL of water and then poured one milliliter of that tube into the 3rd tube. I repeated this step with the wild-type.

Part 3: UV Exposure

Finally, I set up the exposure growth plates. I made a growth environment with 2 grams of growth agar and 200 mL of distilled water to pour into twenty plates that would be exposed to UV radiation (2 controls). Then, for the concentration of each type, 3 plates were set up for one, five and ten minute intervals. Therefore, eighteen plates were made with the growth agar and a 100 microliter sample of the specific concentration of a spore type. Then, I exposed these samples to the UV light box under germicidal UV for their labeled time frames with the dish covers off and allowed them to either grow or die overnight in a drawer at room temperature with parafilm around the edges. I then analyzed these results by relating the amount of melanin to the amount of growth versus death of the spores because the protective nature of melanin in the two types of the fungus is

shown through the growth and death rates.

RESULTS:

These results are depicted in the data table and graph. The wild-type grew more colonies than the tan-type at the same time intervals. The higher concentrations of spores of each type grew more colonies than the lower concentrations at the same time intervals. The tan-type was more likely to die under the UV light at the times compared to the wild-type.

Figure 4. UV light exposure box with samples and lab setup.

DISCUSSION:

The conclusion that can be drawn based on this experiment is that the wild-type fungus is more resistant to spore death under UV light than is the tan-type. The wild-type spores were able to grow more colonies after exposures at the same time intervals. This means that my hypothesis is likely to be correct: having darker skin pigmentation allows humans to have a biological advantage and resist UV damage and skin cancer. The connection between a higher amount of melanin and resistance to UV damage

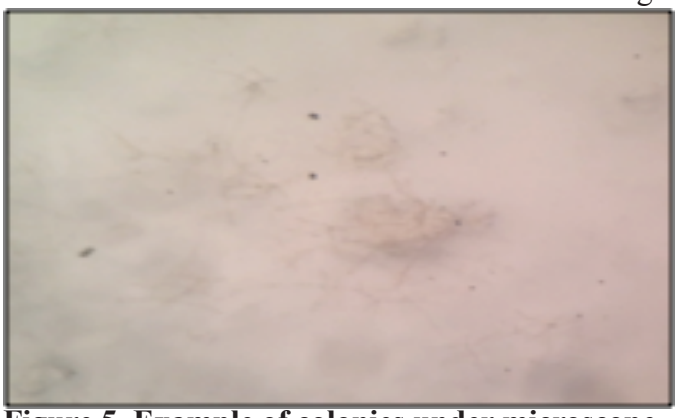
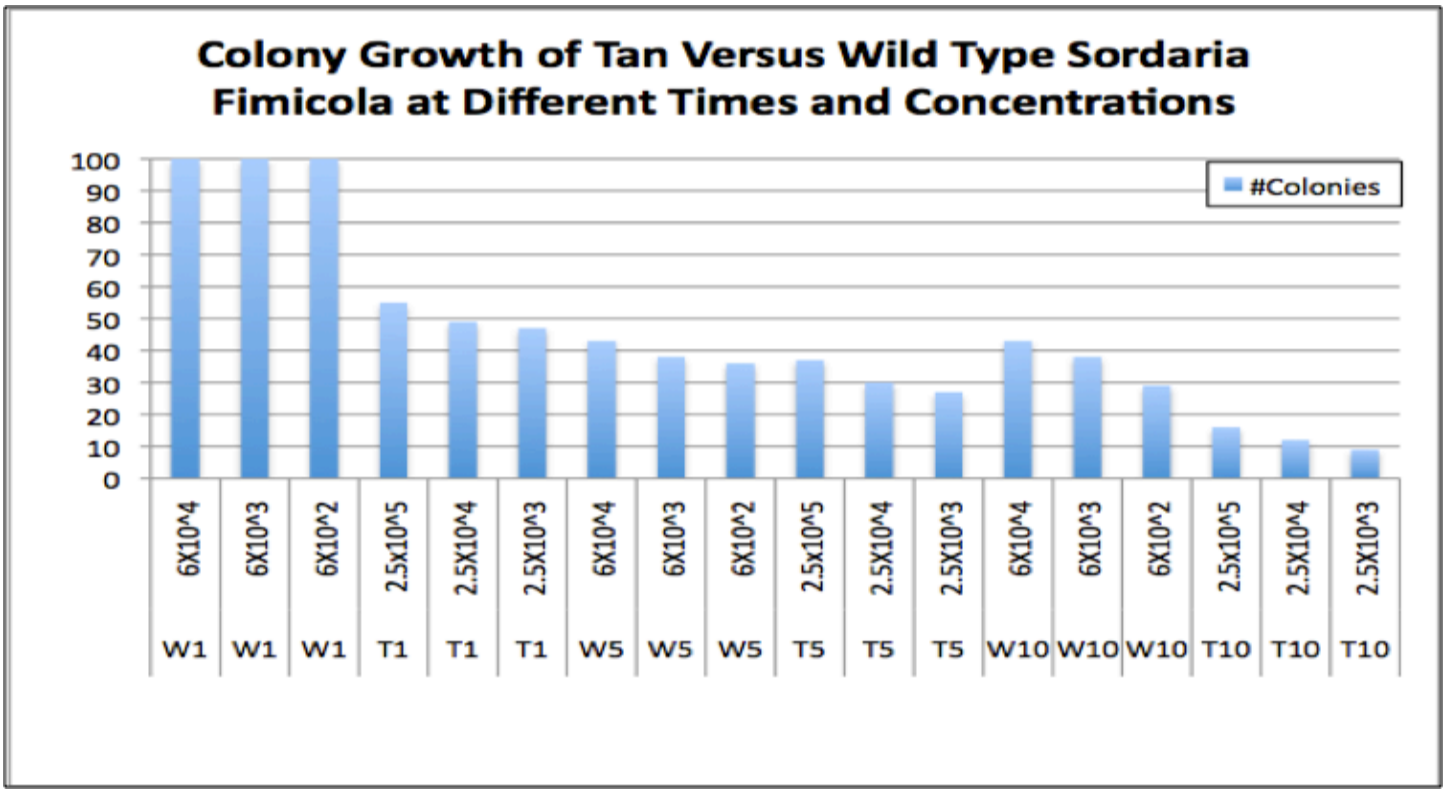


Figure 5. Example of colonies under microscope.



is now clearer, as there is a photoprotective element to melanin levels. Yet, to strengthen this conclusion I would repeat the experiment with more samples and expose them for more time to find a point at which colony growth stops. I would test positive controls for each type of fungus as well and use a third gray-type. Overall, experiment was successful because the results and observations further supported my hypothesis.

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Plasmid Construction for a Zebrafish Model of Huntington's Disease

By Sonalika Reddi'13 and Amanda Haik'13

ABSTRACT

The goal of this project was to construct plasmids that would subsequently be injected into zebra fish (*Danio rerio*) to give them Huntington's disease. Once plasmid incorporation into the fish's genome is successful, the next steps in the project would be to test the Huntington's phenotype in the fish and knock down certain proteins, such as Rhes, to see if symptoms are then alleviated. During this plasmid construction, a series of protocols were necessary to construct three plasmids: zsgreen, wild-type Huntington's, and mutated Huntington's. In the end, each plasmid had an ubiquitin promoter, green/wtHtt/mHtt, and the BGH terminator. The project culminated with the beginning steps successfully executed for plasmid design.

INTRODUCTION

Huntington's disease (HD) is a genetic disorder in which nerve cells in certain parts of the brain progressively degenerate. It is caused by a polyglutamine repeat in the wild-type protein (wtHtt), which results in the protein mis-folding. As a result, the protein becomes mutated and is expressed throughout the body and in all regions of the brain, causing Huntington's disease. The disease is neurodegenerative and results in uncontrolled movements such as sudden jerking and facial movements, and behavioral and psychological changes like hallucinations and loss of memory. Huntington's effects eventually progress and become more pronounced as time goes on (1).

Rhes, a striatal-specific protein, is known to increase the toxicity of HD. It breaks the mHtt protein up into smaller clumps, which can easily slip into the nucleus and increase toxicity. There is speculation that by knocking down Rhes, some of the HD symptoms will be alleviated, as this will eliminate Rhes's ability to enhance mHtt toxicity (3).

In order to test the effect of Rhes on Htt toxicity, we first have to establish a baseline of HD symptoms to compare. This control is important so when Rhes is knocked down, any symptom changes in the phenotype can be observed. Since human testing is not yet viable, zebra fish (*Danio rerio*) are a good alternative in a high school environment. They are often used for scientific research particularly because of their close homology to humans,

providing for a way to investigate human diseases such as HD.

The objective was to make transgenic zebra fish express the mHtt protein to then test them for the Htt phenotype. We will use plasmids, circular pieces of DNA that can be transformed into the genome, to inject into the embryos of zebra fish. Through PCR, digestion, and ligation, specific sequences of DNA can be put together in a unique plasmid that can be successfully incorporated into the zebra fish genome. Therefore, for our project this year we set up the plasmids that would ultimately be injected into the zebra fish to give them HD. Three plasmids were required: green plasmid (for marking purposes and to increase the efficiency that the fish will successfully incorporate the Htt into their genome), wtHtt plasmid, and mHtt plasmid. Each plasmid will have a promoter, the green/wtHtt/mHtt, and an ubiquitin terminator.

MATERIALS AND METHODS

We must design three different plasmids: zsgreen, wtHtt, and mHtt. We thus began the experiment by first designing primers. We went back to the vectors we were using (pzsgreen, Pentr5'_ubi, and Pex100t) and looked at the respective multiple cloning regions (MCR). We used pzsgreen because it encodes a variant of green fluorescent protein (GFP), which we need to ultimately target the fish later on in the experiment. We used the pentr5'_ubi vector because it encodes for the ubiquitin promoter, which is universal in all cells of the body. Ubiquitin expresses transgenes at all developmental stages (4). Finally, we used the pex100t vector because it contains the meganuclease sites, which help increase the efficiency of the plasmid actually being incorporated into the genome of the zebra fish.

We then selected two restriction enzymes located in each MCR to then determine where we can put overhangs on each respective primer. Overhangs enable the primers to lie down on DNA. The primers must have two different restriction sites on the target vector which allow the insert to be put in the right spot, and the restriction sites cannot be located internally on the insert to avoid cutting in the middle of our target sequence. Primers should also be around 25 base pairs long with roughly about 50% G-C content and melting temperatures near 59 degrees Celsius.

The primers designed for our project were: BGHpa (forward and reverse) (BGH is the terminator that comes from Htt), with restriction sites ecorI and apaI; Htt forward and BGHpa reverse, with restriction sites ndeI and ecorV; zsgreen forward and BGHpa reverse, with restriction site bamhI; ubiquitin promoter forward and BGHpa reverse, with restriction site for pex100t; and pex100t to test the final plasmid.

After the primers were designed, the next step was to run PCR, which amplifies a target sequence of DNA, on the BGHpA terminator from the Htt template. We used the PCR protocol for Phusion Hot Start DNA polymerase (5), and followed up with a gel electrophoresis and an analysis of band length to confirm success of the PCR. Since the gel was successful, we then digested the PCR product and green vector with appropriate restriction enzymes, using the restriction enzyme protocols from New England BioLabs. During the digest, we dephosphorylated the vector by adding Cip. It is important to dephosphorylate the vector so it does not just reattach to itself. We followed up with another gel to make sure the digestion was successful. The next step was running a PCR purification protocol. This was important, since PCR amplification products must be purified from undesirable reaction components such as primers, enzymes, and salts. The next step was ligating the BGHpA insert into the green vector, followed by a rapid colony transformation, and overnight and miniprep colonies on a plate. The final step of this phase was sequencing zsgreen to confirm it had the terminator.

We then proceeded with PCR of zsgreen + term, wtHtt + term, and mHtt + term, and then repeated above but into ubi plasmid, which had the ubiquitin promoter. Then we used PCR with promoter+zsgreen+BGH, promoter+wtHtt+BGH, and promoter+mHtt+BGH and repeated above but into pex100t plasmid, which had the Iscei meganuclease sites. This resulted in three plasmids.

DISCUSSION

Our PCR for the wtHtt+BGH and the mHtt+BGH were successful. In Figure A, very clear bands are visible in those two lanes. However, the PCR for the BGH was unsuccessful since no band was visible in the lane at the 227 bp mark. A possible explanation for the failure is DNA fragment size, which was too small for a successful PCR. In order to remedy this problem, we tried increasing the cycles on the PCR machine in order to get a higher quantity of PCR sample to then run on the gel. We then also tried using a .75g agarose gel rather than a .5g

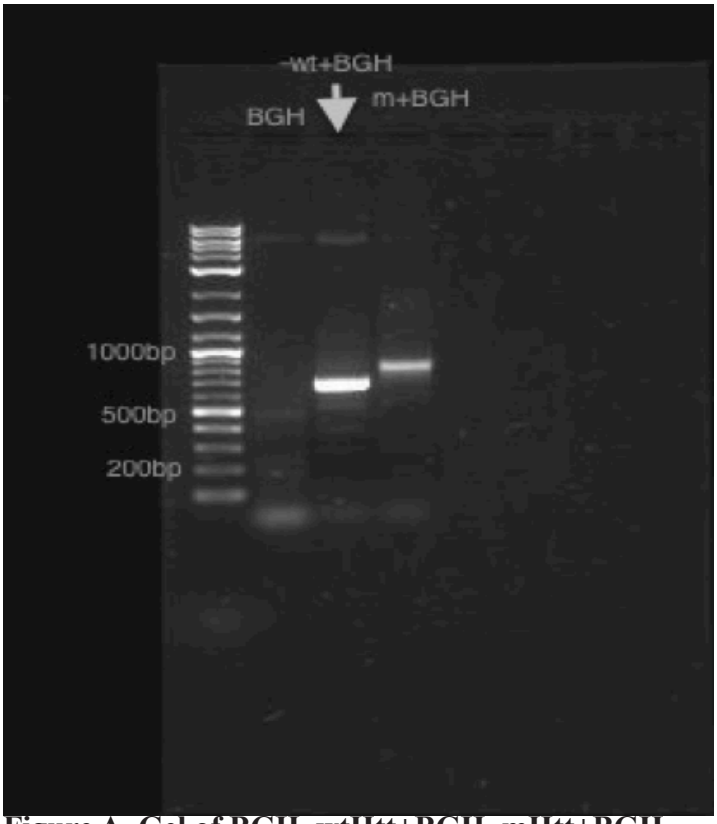

Figure A. Gel of BGH, wtHtt+BGH, mHtt+BGH PCRs
We received successful results with the wt+BGH and m+BGH with very clear and bright bands. The BGH band, however, is not so clear. There could possibly be a band at 227 base pairs, but that may simply be a primer.

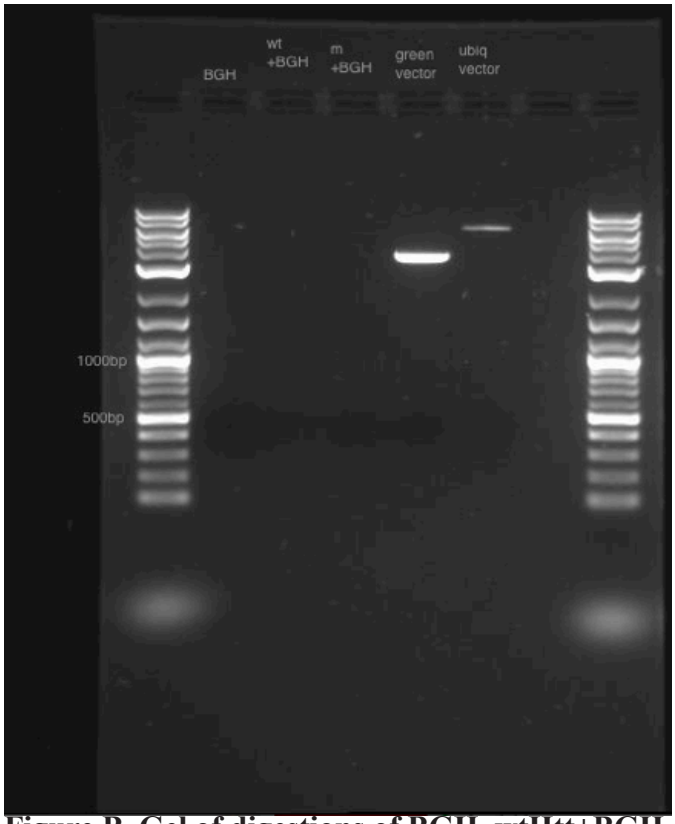

Figure B. Gel of digestions of BGH, wtHtt+BGH, mHtt+BGH, green vector, and ubiquitin (ubiq) vector
The green and ubiq vector digestions were successful since there are bands in those lanes. However, the digestions for the BGH, wt+BGH, and m+BGH were unsuccessful since there are no bands. If successful, there must be two bands in each lane, showing the cuts from the enzymes.

agarose gel. The increased amount of agarose would hopefully slow down the movement of the DNA fragment so it would successfully appear on the gel. However, none of these changes worked, as the BGH PCR remained unsuccessful. On the other hand, the PCR reactions of the wtHtt+BGH and mHtt+BGH were successful, which shows that we executed the protocol successfully and it was most likely the size of the fragment that caused the BGH to not work alone.

The next phase of the project was digestion. Based on gel analysis as seen in Figure B, we can see that the digestions of the two vectors (last two lanes) — ubiquitin and green — were successful, whereas those of the three PCR products were not. We should have seen two bands in each of the first three lanes to represent the digestions of the PCR. We ran the BGH PCR through the digestion protocol even though we never received successful PCR results because maybe some of the blurred bands in the gel could have been successful results rather than just primer. But with no results in the digestion, it is likely that we never had BGH PCR results to begin with like we initially thought. Also, since two out of five digestions were successful, we can assume that the errors did not lie in carrying out the protocols, but it is possible. Another source of error lies in the possibility that the enzymes could have denatured in the time period when we were fixing the faulty water bath. However, it was also unlikely that only some of the enzymes denatured. The inconsistency in the results made this difficult, especially since in theory the wtHtt+BGH and mHtt+BGH PCR digestions should have worked since we got successful PCR results from the first step. Nonetheless, at this point we ran out of time to continue troubleshooting the project.

CONCLUSION

Assuming successful results at this point in the project, the future steps would be ligation,

followed by a bacterial culture. Then we would repeat these steps by putting each of the inserts into the pex100t vector. The plasmids must subsequently be microinjected into the zebra fish embryos, which would then be allowed to develop for three months. Once the fish have grown, the next step of the project would be to test the Huntington's phenotype with a series of cognitive tests in the fish. After establishing a baseline, we would knock down Rhes, an elemental protein in creating Huntingtin (the Huntington's protein) aggregates, to see if symptoms are alleviated.

ACKNOWLEDGEMENTS

We would like to thank Michaela Ennis, who initially designed this project. She also continually helped us along the way with any and all questions. We would also like to thank Mr. David Maxwell for giving us the opportunity and time to work on such a great project. We also want to thank Mr. Luke De and Dr. Morgan Thompson for supporting us and helping us with any questions or problems we encountered throughout the year.

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Factors Influencing Healing Rates of Arthroscopic Repairs of the Rotator Cuff

By Kaitlyn Friedman'13

ABSTRACT

Roughly 200 million dollars are spent in the U.S. annually to treat over four million people with rotator cuff issues (2). However, rotator cuff surgery has a high variability of success. There are many factors that influence healing. In this study I examined factors that might improve success rates. I defined failure as the need for another surgery. I studied 70 consecutive patients operated on by the same surgeon, with an overall success rate of 79%. Through this study, I determined that the most important factors are the tear size and the quality of the tendon. Generally, I suggest that the surgeon increase the number of anchor points as the tear size increases or as the quality of the tendon decreases.

INTRODUCTION

Published studies believe the success rate of rotator cuff surgeries to be anywhere between 11% and 80% (3). There are many factors that influence healing. The purpose of this project was to evaluate and determine which factors influence healing and subsequent success. Hopefully, identifying how these factors affect the outcome will allow surgeons to better prepare patients and possibly modify their techniques to improve the success rate. I did this by observing surgery, keeping a data acquisition sheet for each patient and assessing if any or all of these factors influence the healing in terms of strength, mobility, and reduction of pain.

So what are the factors that influence healing? Some include the size of the tear, the quality of the tissues and bones, the type of repair, smoking history, age, and other co-morbidities, such as diabetes (2).

The rotator cuff is a collection of muscles that act on the glenohumeral joint, or shoulder joint, which are used to rotate the arm. There are four muscles that make up the rotator cuff: the Supraspinatus, Infraspinatus, Teres Minor and Subscapularis (4). A rotator cuff tear may result from an acute injury, such as a fall, or by chronic wear and tear with degeneration of the tendon. When tearing occurs, the best strategy to fix it is through a surgical procedure

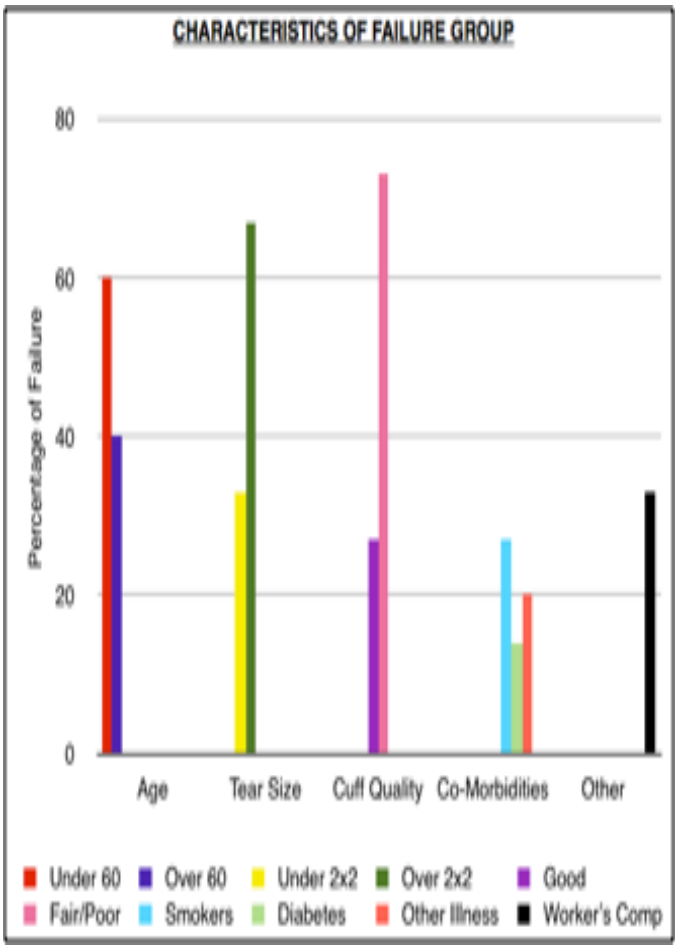
(1). Surgery involves reattaching the torn tendon, in the hope of regaining strength and mobility and reducing pain.

MATERIALS AND METHODS

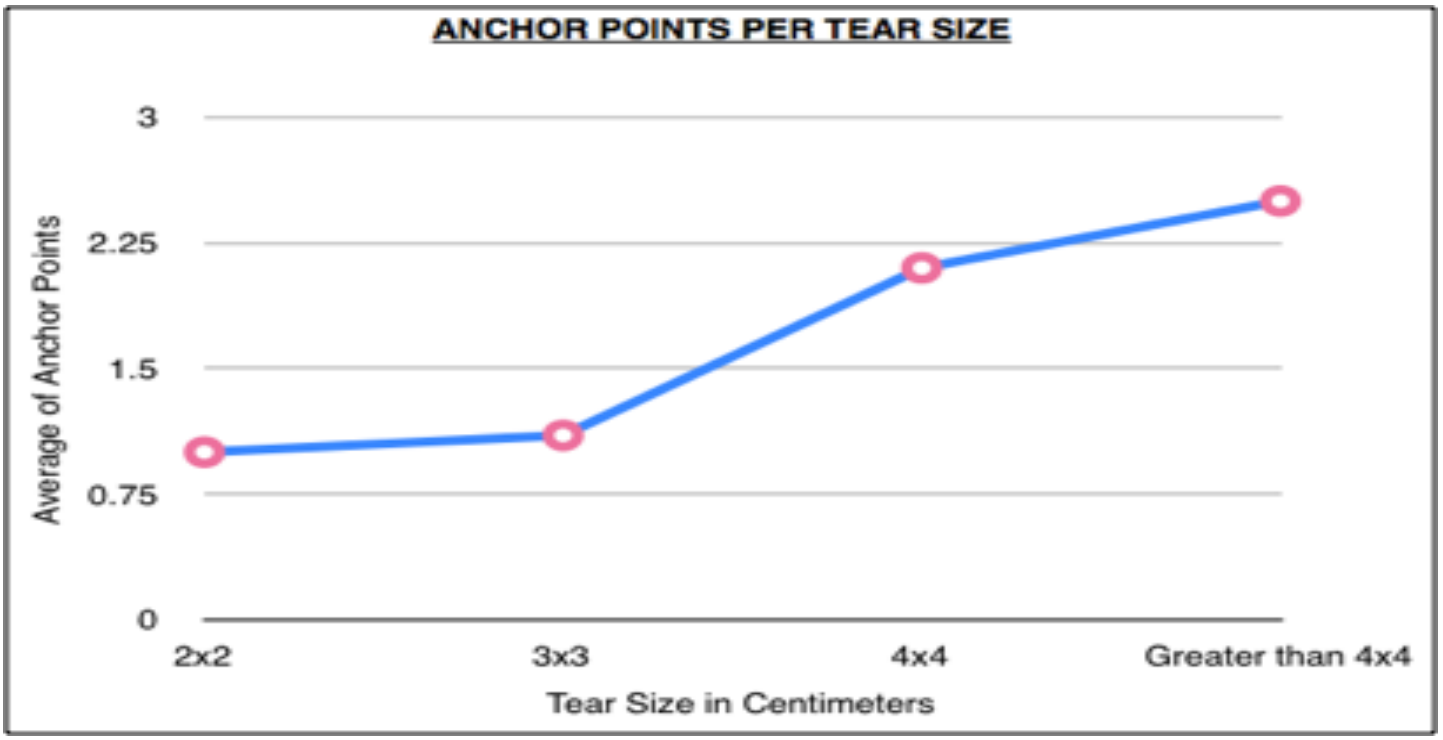
I began this project by observing standard rotator cuff surgery, to better my understanding of the procedure. For each patient I received signed consent before observing surgery or looking at his or her records. I compiled data on each patient, and followed up with the patients' routine check – ups.

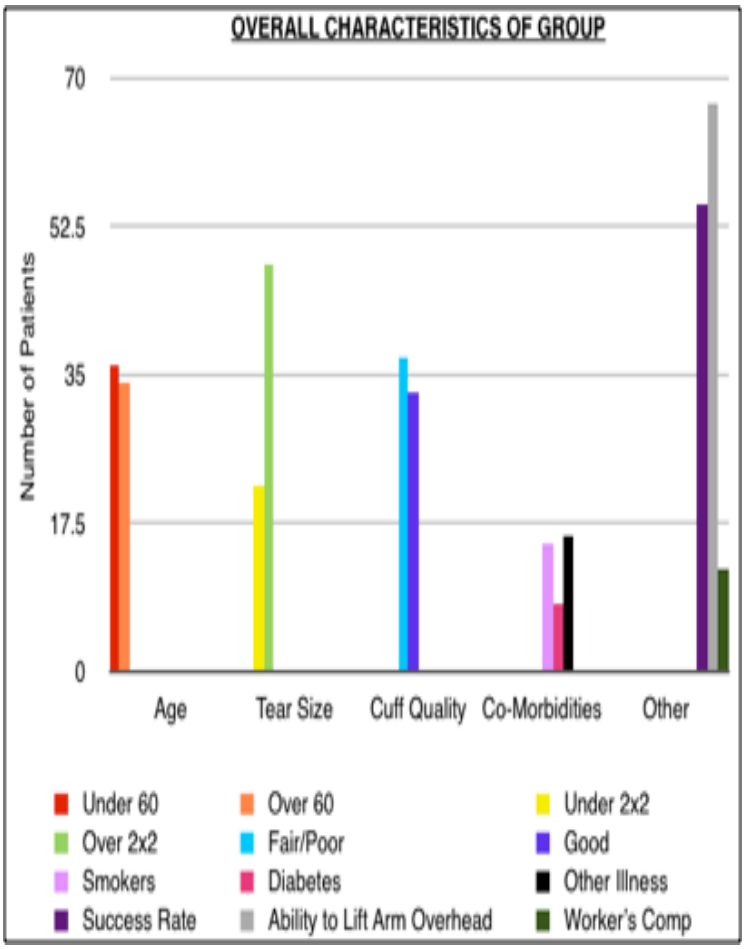
The data I collected included basic information regarding the patient's age, gender, date of surgery, and more specific information including tear size, rotator cuff quality, type of repair, and associated co-morbidities. The patient gave me basic information and the doctor noted specific information. Then I collected data including reduction of pain, ability to lift the arm overhead, and the need for more surgery. Whether or not the case was Worker's Compensation was also noted, due to the tendency for patients to exaggerate pain when getting out of work. I took information on seventy patients, including all patients who allowed me review their files and give me enough time to see some, if not all, of their rehabilitation.

I then analyzed the collected data and tested to see if there were relationships between these factors and the success of the surgery, and if the relationships were statistically significant.



RESULTS





DISCUSSION

The overall success rate for this series of patients is 79% with a group of relatively large tears. Of the 15 failures, 12 had more surgery, and half of those achieved success, increasing the overall success rate to 88%.

A trend was identified in which the tear size and tendon quality negatively affected the results. Larger tear sizes and poorer quality tissue were harder to repair.

Co-morbidities, such as diabetes, and smoking did not seem to affect the results. This may be due to relatively small sample size.

As the area of the tear increased, the number of anchors used only slightly increased. Given that the larger percentage of failures occurred in the large tear group, it is recommended from this study that the surgeon increase the number of anchor points as the tear size increases:

4 cm2 (2 X 2): 1 anchor point

9 cm2 (3 X 3): 2 anchor points

16 cm2 (4 X 4): 3 anchor points

>16cm2 (4 X 4): 4 anchor points

Each anchor point costs $250, so there is pressure on the surgeon to decrease the number of anchors. However, another surgery costs more than adding one or two additional anchors.

If poor tissue quality is found, it is recommended that the surgeon increase the anchor points as well.

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The Effects of Exercise on Short-Term Cognitive Ability

Mac Hugin'13 and Jack Wollmuth'13

ABSTRACT

When someone takes part in physical exercise their brain releases chemicals in response to this exercise. These chemicals, neurotransmitters, give off a "high" that not only increases mood and decreases anxiety, but also increases short-term cognitive ability. Epinephrine is the chemical that creates an adrenaline rush when one exercises. This chemical not only gears the body up for exercise, but also gives the body a good feeling during and after exercise (2). Serotonin is the main neurotransmitter that elevates mood such as satisfaction, but is also depleted by stress, anxiety, and inactivity (3). Endorphins are the neurotransmitters that act as natural painkillers. These chemicals reduce pain during and after exercising. Endorphins are also partially responsible for the "high" that we experience after exercising. Dopamine is the neurotransmitter responsible for sleeping and waking cycles. Dopamine, like serotonin, is depleted by stress and anxiety, but elevating serotonin levels can elevate dopamine levels. Exercise causes the release of neurotransmitters that positively affect the body's functions (2). The level of each neurotransmitter that is released can vary depending on the intensity of exercise, therefore affecting the amount of increase in cognitive ability.

INTRODUCTION

It is known that one's short-term cognitive ability is positively influenced by exercise (1). We have studied the effect of varying intensity of exercise on male teenage hockey players, and kept as many underlying factors constant. We administered a series of cognitive tests before and after each exercise. Our objective was to quantify which type of exercise most positively influences the cognitive ability of teenage males. Testing both aerobic and anaerobic exercises, continuous vs. high intensity intervals, allowed us to draw relationships between cognitive ability and exercise, across both interval and continuous training (1), which would lead to an ideal workout to improve short-term cognitive ability of in season teenage male hockey players. Because in season hockey players train at high intensity intervals, our hypothesis was that they would be more positively affected by continuous training since their body is not used to specific training, causing them to release an increased amount of neurotransmitters.

MATERIALS AND METHODS

Twenty male ice hockey players between the ages of fifteen and eighteen participated in the experiment (ten for interval training and ten for continuous). Pre and Post workout cognitive testing was conducted in a room with each subject working on a separate desktop computer. Researchers monitored the subjects during all testing to ensure accuracy and provided instructions for each test. Subjects took four tests, that have been proven to be an accurate means of measuring reasoning, concentration, mental agility, and short-term memory recall. Three tests were provided by a website called Cambridge Brain Sciences (4). The three Cambridge Brain Sciences tests are Grammatical Reasoning, in which subjects had one minute and thirty seconds to answer as many true or false questions correctly as possible, Spacial Span, in which subjects had three tries to get the highest possible score, and Feature Match, in which subjects had one minute and thirty seconds to answer as many questions correctly as possible. All Cambridge Brain Sciences tests give scores depending on the subject's performance (4). The fourth test was based on a well-documented psychological phenomenon known as the Stroop Effect. Subjects were timed on how fast they could complete the test with accuracy (5). All four tests produce scores to easily quantify the increase in cognitive ability after exercise.

In order to test if exercise improved subjects' cognitive performance, subjects took each test prior to exercising, and then took each test after completing their workout. Subjects were supervised by one or both researchers while taking the tests and were also supervised while they exercised. After either running for twenty minutes at a continuous ten minute mile or undergoing interval training,

subjects then took the tests again after a five minute rest and the data was compared to previous scores in order to determine whether exercise improved cognitive function of subjects.

The same methods were also used to determine the effects of interval training as opposed to those of continuous training. The interval training consisted of 100 meters, full speed sprints, and then a slow jog for 200 meters. Subjects who underwent interval training only exercised for ten minutes. Two trials for both running tests were conducted and took place outside on a standard track.

RESULTS

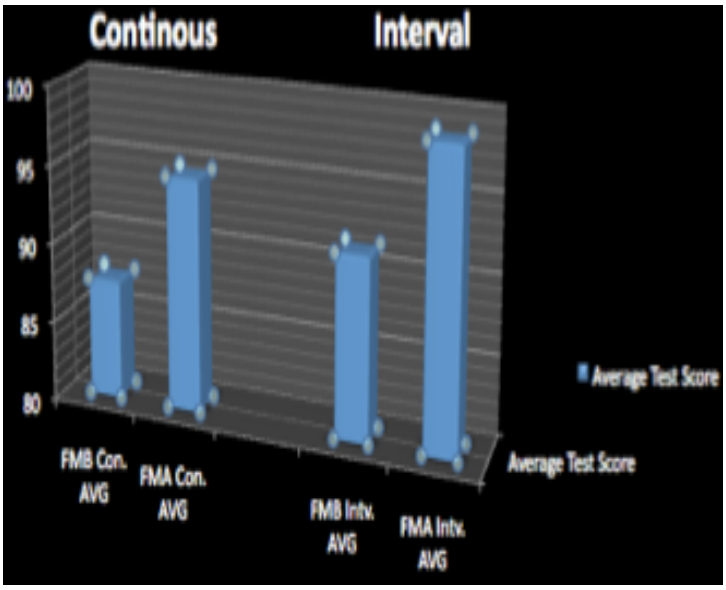

Figure A: Average Feature Match score of subjects before and after interval training versus average Feature Match score of subjects before and after continuous training

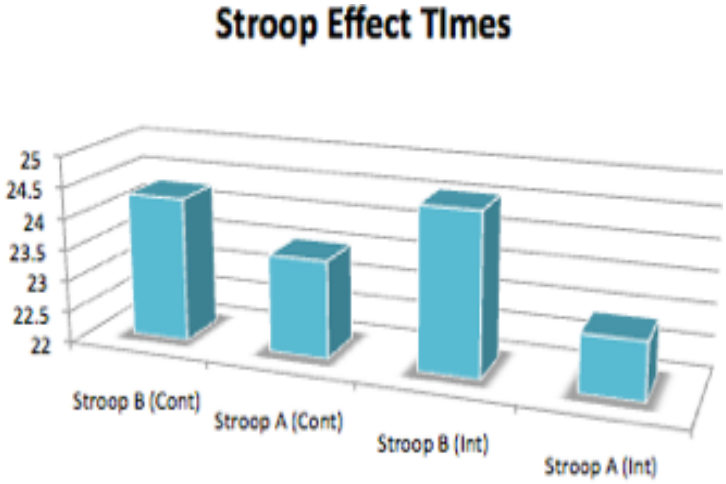

Figure B: Average Stroop Effect score of subjects before and after continuous training versus average Stroop Effect score of subjects before and after interval training

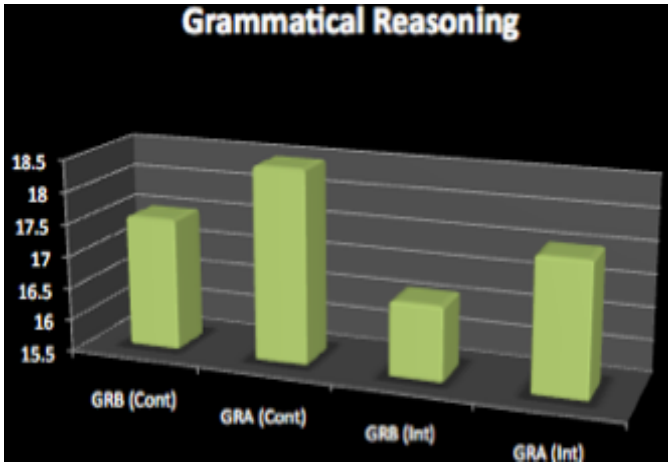

Figure C: Average Grammatical Reasoning score of subjects before and after continuous training versus average Grammatical Reasoning score of subjects before and after interval training

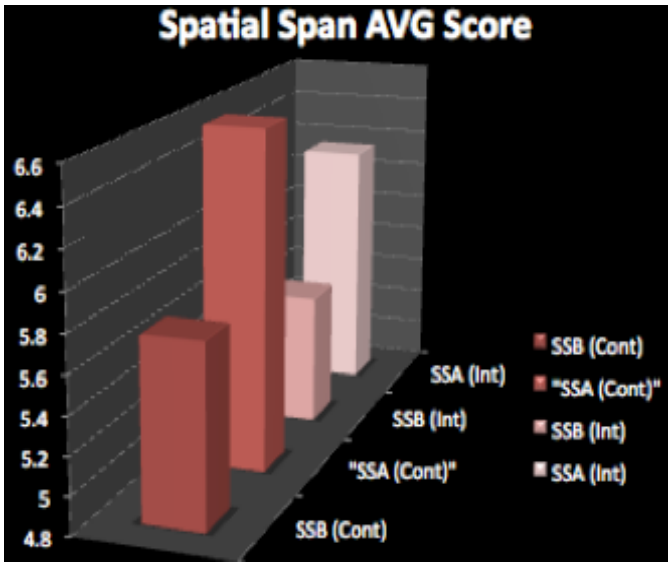

Figure D: Average Spatial Span score of subjects before and after continuous training versus average Spatial Span score of subjects before and after interval training

In the Feature Match, continuous training subjects saw an average increase of 7.2 points (7.6%) and interval-training subjects saw an average increase of 7.3 points (7.7%). In the Stroop Effect continuous training subjects saw an average increase in speed of .75 seconds (3.1%), whereas the interval-training subjects saw an average increase in speed of 1.6 seconds (6.5%). In the Grammatical Reasoning test continuous training subjects saw an average increase of .95 points (5.1%), whereas interval-training subjects saw an average increase of .9 points (5.2%). In the Spatial Span test continuous training subjects saw an average increase of

.85 points, whereas the interval-training subjects saw an average increase of .65.

DISCUSSION

The data emphasizes that short-term cognitive ability is improved by exercise, though fails to show a significant statistical difference between interval training and continuous training. Although our data is inconclusive in proving that continuous training increases short term cognitive ability better than that of interval training for season teenage male hockey players, our results are consistent with the general conclusion that exercise increases short-term cognitive ability. On average, for both interval and continuous training, subjects saw an increase in each category of cognitive function: reasoning, concentration, mental agility, and short-term memory recall.

There is a wide-range of possibilities for why the data did not support the original hypothesis. We should have taken many more trials to eliminate possible outliers and minor experimental error, but subject availability was an issue. Another factor that has to be taken into account is the possibility of subjects getting better at the tests over time. The earlier test scores may be deflated as a result of the subjects not understanding the tests as well as they did towards the end of the experiment. Unfortunately, this means that some of the increase scores may be attributed to the understanding of the tests rather than the increased cognitive ability because of exercise.

In the future the research could be expanded to encompass a larger age group and both genders. A larger pool of subjects and far more trials to ultimately prove statistical significance would also supplement the research. The research could also be improved by testing a wider-range of intensities of exercise. This research would lead to finding ideal intensity exercises to most cognitively benefit subjects of specific gender, age, sport etc.

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We would like to personally thank Mr. De and Mr. Maxwell for their continued support and mentorship throughout the entire project. We would also like to acknowledge the subjects that participated in the testing.

Nepetalactone: The Creation of a Natural Insect Repellent

By Dan Vaysberg’13 and John-Tod Surgeon’13

ABSTRACT

The catnip (*Nepeta cataria*) plant has been used for centuries for medicinal purposes in humans and most recently for recreational purposes in some felines. The one thing that has been newly discovered about catnip is that it can be used to repel mosquitoes. With widespread mosquito-transmitted diseases and no chemical barrier to thwart the transmission, catnip offers a cost effective way to save lives.

INTRODUCTION

Mosquitoes are a blight and nuisance for practically every society on the planet. Mosquitoes cause over 600,000 deaths over the course of a year through various infectious diseases. A cheap, safe, and effective way of repelling mosquitoes could drastically reduce the more than 200 million cases every year. Our current defense against mosquitoes is a chemical called DEET. Although effective, research shows that in high quantities DEET can cause cancer and induce seizures. We researched a safer and possibly more cost efficient, repellent. After researching on our own and having an inside source, we learned of a chemical compound known as nepetalactone that may serve as a viable mosquito repellant. We extracted the nepetalactone from the leaves of the catnip plant and then we subjected it to two different types of tests to ascertain its repellent strength. In the test, Fruit Flies were used in place of mosquitoes because fruit flies are not only very similar to mosquitoes, but also are safer for the test subjects. After extracting the compound we ran tests on both fruit and human skin. (Results discussed later)

MATERIALS AND PROCEDURE

For the materials in the experiment we used catnip leaves, water, mortar and pestle, cooler, ice, clear rubber tube, stovetop teapot for the extraction of the nepetalactone. When testing its effectiveness as a fruit fly repellent, we used a dual exit enclosure, a glass box with and armhole, fruit, fruit flies.

The procedure for distillation is as follows:

Take glass stovetop teapot, place evaporation tube in steam hole, fill with 100ml distilled water; bruise 28g (1oz) of catnip leaves in a mortar, insert the bruised catnip into the teapot, run evaporation tube through empty igloo cooler with end coming out of the drainage hole; fill cooler to the brim with ice and water (make sure the hose is snugly in the hole to avoid water contamination of the nepetalactone), again to avoid water contamination attach a small clear balloon at the end of the hose as the collection vessel; place teapot with catnip water mixture on the stove, and heat at 72 °C for 25 min. Repeat until you have produced enough of the distilled oil.

The procedure for testing is as follows:

Prep two pieces of fruit. One goes unaltered into a clear cylindrical container, which is then attached to a T PVC joint pipe. The other is soaked in the nepetalactone oil for 20 min, then placed in a different clear cylindrical container but attached to the other side of the same T PVC joint pipe. Fruit fly culture is introduced by attaching their cylindrical container to the third end of the joint. Let rest upright and observe, counting the amount of times the flies come in contact with fruit in the 10min allotted time.

The second procedure for testing is as follows:

Take a glass tank with an opening at the top, cut out a cardboard rectangle to fit in the frame of the opening, attach to the tank on only one side (to allow release of the fruit flies); cut one hole 10cm in diameter in the center of the cardboard, cover hole on the outside with cling wrap with tape (to prevent the escape of the flies), insert flies into the tank; cover arm with sucrose, insert arm (breaking cling wrap) observe the frequency the flies land on skin over a 10 min period; repeat 2 more times, then repeat 3 times with the addition of nepetalactone to the arm along with sucrose.

RESULTS

The fruit flies were found to be less likely to land on nepetalactone-covered surfaces. This "repellent action" was only maintained for, at most, 5 to 7 minutes. On warmer surfaces, the results were less demonstrative of the overall conclusion because the time in which the nepetalactone was effective at repelling was reduced significantly.

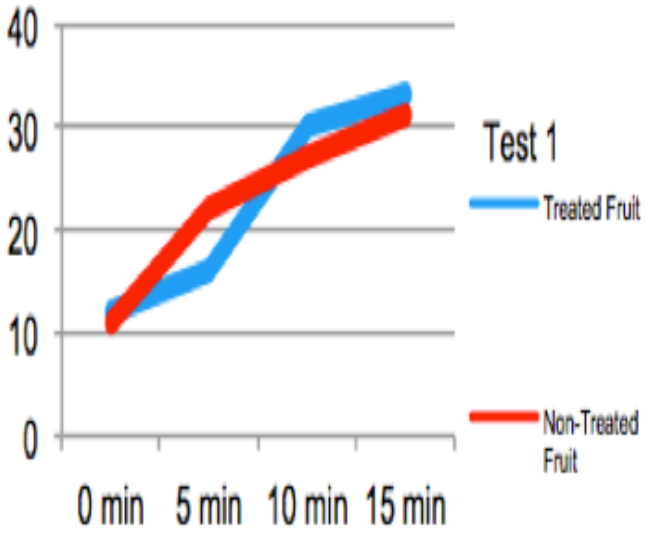

Fig. 1

The results shown in this graph and in Fig. 2 show the quantity of fruit fly touches on the fruit. In the first test, the amount of flies that were attracted to the nepetalactone treated fruit equaled the amount attracted to the non-treated fruit.

THE FRUIT TEST PART I

The first fruit test was run originally three times, and in those three trials an amount of flies were assessed at specific time intervals. The raw data at the time intervals were then made into an average amount for that specific time. In the first set of three trials for the fruit test, the data showed that the flies were not significantly affected by the nepetalactone. For the first five minutes of this test the results show that there is nothing but slight variation between the non-treated fruit and the fruit treated with nepetalactone. Also. this variation was deemed statistically insignificant; so for all intents and purposes, the treated fruit was as ineffective as the non-treated fruit at repelling fruit flies.

THE FRUIT FLY TEST PART II

The results in the first set of three fruit test trials varied greatly from our original hypothesis. We believed that the discord between the data and the hypothesis was due to human error in the extraction (of the nepetalactone) process. Because of this belief we decided to redo the extraction process, and also redo the set of three fruit fly test trials. In the retest, flies were more likely to land on the fruit that was not treated with nepetalactone (for the first five minutes). After the five-minute mark the number of contact incidence between the flies and the nepetalactone saturated fruit steadily increased. By the end of the testing session of 15 minutes the amount of flies touching the treated fruit escalating at the same rate as the non-treated fruit.

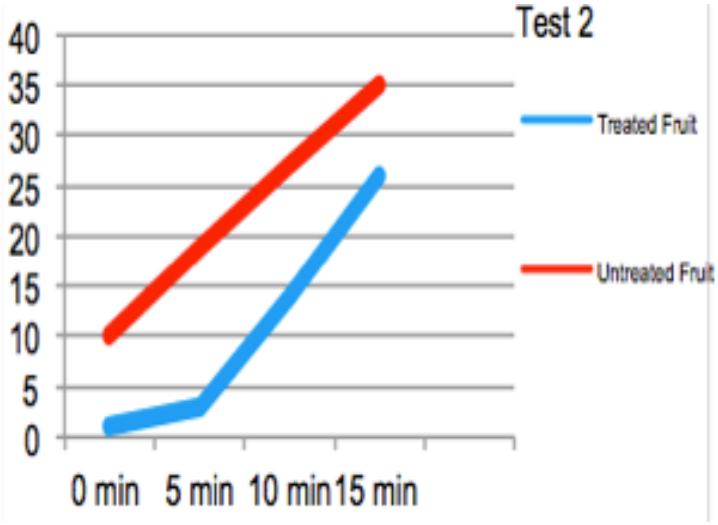

Fig. 2 The experiment was re done, using new nepetalactone extract.
In the second test the results held to our hypothesis that for a short period of time the nepetalactone would repel the flies effectively.

THE SKIN TEST

The skin test's purpose was to test the effectiveness and the practicality of the application of nepetalactone on human skin. This test's results were inconclusive, because just like the first trial of the fruit test the results from the non-treated arm had a variation that was statistically insignificant from the results from the nepetalactone treated arm. The reason why the results were not statistically significant in variation is for two different reasons. The first reason is that we were not able to conduct more than two trials for this test due to the flies' death rate and their ability to escape their containment vessel we were running low on fruit flies. The second reason is that the ambient surface heat of human skin is much higher than the room temperature piece of fruit. This does not allow us enough time to properly record and observe the repelling period.

DISCUSSION

THE FRUIT FLY TEST

In this test the results tell us a few things, the first thing that the results tell us is that with diluted nepetalactone the potency of the repellent is diminished. The second thing established by the data is that nepetalactone at room temperature is not very effective at long-term repelling. This makes sense because nepetalactone is an Esther, which would mean that is very volatile. Its volatility allows it to evaporate at low temperatures, making it easy to extract from a water solution, but hard to stabilize on a surface. This trait makes nepetalactone in its natural state very impractical when it comes to real-world application of any kind.

THE SKIN TEST

This test only furthered the realization that nepetalactone is not practical in its natural form for a real-world application on human skin. The results show that at no time did the nepetalactone treated arm have any enhanced repelling power over the non-treated arm. From the original time marking there was no statistically different variation between the two arms and as the time marking progressed the rate at which fruit flies were touching both arms increased similarly. We have good

reason to believe that since the temperature on the skin is much higher than room temperature the speed at which nepetalactone will evaporate would be faster, and that would explain the decrease in repelling efficiency.

CONCLUSION

In the future a continuation of this research would need to address the problems in practicality. The purpose of this project was to produce a product that would serve the greater world community in an effort to thwart the silent killer, malaria. In its current state nepetalactone shows promise in that regard, but because of its volatility it is not yet ready to serve the purpose that the world needs. To continue this research is to continue the betterment of our society. This research will inevitably produce a chemical barrier between humans and mosquitoes, saving lives in the process. Our results are quite conclusive and they indicate that nepetalactone is a ligament way of repelling fruit flies and by extension mosquitoes. Something to move forward thinking about is the idea of stabilizing the substance in an oil-based cream. With the added prospects of an all-natural substance that repels mosquitoes this product could potentially change the course of history. With more advanced equipment all of these things can be possible and nepetalactone will be able to dramatically diminish the amount of malaria deaths and infections on per year basis.

ACKNOWLEDGEMENTS

Special Thanks To: Dr. Lauren Harrison, Hoffmann-La Roche Inc., Mr. David Maxwell, The Pingry School

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Correlation Between Fish-Feed and Quality of Their Feces as Fertilizer for Fast Plants

By Emily Kamen'13 and Dan Muro'13

ABSTRACT

The goal of our research project was to determine whether the feed given to zebra fish affects the quality of the fishes' feces as a fertilizer for plants. We planned to do this by setting up our own aquaponic system and feeding three tanks of fish a type of food similar to their natural feed, brine shrimp, and the other three tanks soybean meal, a cheaper alternative. We hypothesized that feeding the fishes what they would eat in the wild would lead to a fertilizer with more nutrients, which would be more effective and lead to taller plants. Our results did not prove this hypothesis to be true, because we were unable to grow the plants whatsoever due to lack of germination.

INTRODUCTION

Aquaponics is a sustainable food production system that combines aquaculture, the raising of marine animals, with hydroponics, the cultivation of plants in water. This system takes advantage of the nutrients in fish waste (nitrogen, phosphorus and low amounts of potassium) as fertilizer for the plants. The water that the fish are swimming in both waters the plants and provides them with natural, renewable fertilizer. Aquaponics is a sustainable system not only because it self-produces fertilizer, but also because it provides two food sources—the crops and the fish. However, research such as the University of Albany's Institute for Health and the Environment shows that what fish are being fed on farms can lead to increased health risks (1). But why would farmers feed their fish more nutritious food, which is more expensive than less nutritious food such as soybean meals, if both types of foods produced the same quality of plants? Therefore, the goal of our project was to

determine the effect of nutrition on the quality of fertilizer produced from the fish feces. We tested whether fish fed brine shrimp produced a higher quality fertilizer than fish fed soybean meal (2).

MATERIALS AND METHODS

We began by setting up tanks for the fish. The tanks were comprised of a 4L plastic box filled with tap water to about 2cm from the top. We added 50 ml of pebbles from a preexisting fish tank (20 gallons that has contained feeder goldfish for multiple years). The pebbles contain the bacteria necessary to convert ammonia, which fish release in their excrement but cannot tolerate in high concentrations, to nitrites (nitrosomonas), to nitrates (nitrobacter), which is usable for fertilizer. These tanks must be set up a few days before any fish are put in them, for the bacteria to work and prevent the fish from being subjected to large amounts of ammonia, which causes ammonia poisoning.

Once tanks were set up, we added fish to each tank. For an aquaponics experiment, any type of fish can be used. We used zebrafish because they are popular and suitable lab subjects, and because we had limited space and could not house larger fish. We made sure the fish had ample oxygen by placing an oxygen bubbler tube in each tank. We fed the fish in three of the tanks freeze-dried brine shrimp, and fed the fish in the other three tanks soybean meal. To make soybean meal, we acquired dried soybeans and ground them into a powder. We fed fish the same food twice a day from the beginning until the end of the experiment.

Next, we set up the hydroponic system. To do this, we acquired 48 Styrofoam fast-plant quads. First, we put a wick through the bottom of each of the four cells in the quad. Then we filled the quads with soil. We put four fast-plant seeds in each cell. We saturated each cell with water, until they were dripping water out the bottom. We placed a sponge-paper on the lid of the plastic box that covered the entire lid and extended far enough into the tank that water can be absorbed from the tank onto the paper. We placed 8 quads filled with planted fast-plants on each lid, and placed lids on top of the tanks. We monitored the growth of plants daily by recording the height of the plants.

To set up controls for the experiment, we planted three additional quads. We watered one of the fast-plant quads with water that is from a container that has a proportionate amount of tank-pebbles, but does not receive food. We watered the second quad with water sourced from a container that had a proportionate amount of tank pebbles and soybean meal in it and the third quad with water that was from a container that has a proportionate amount of pebbles and freeze dried brine shrimp in the water. We monitored the growth of these plants in the same fashion as the aquaponic systems.

RESULTS

We did not have sufficient data to observe a difference in the height of plants being fertilized by water from the tanks of goldfish being fed soybean meal and water from the tanks of goldfish being fed brine-shrimp. Our fast plants did not germinate, and therefore there was no plant growth. One cell of a quad being fertilized by a tank with brine-shrimp-eating fish tank grew a green mold.

DISCUSSION

Unfortunately, because our fast plants did not germinate, we were unable to determine whether type of food fed to zebra fish/goldfish can affect the quality of the fertilizer for fast plants—measured by their height. We believe that the plants did not germinate because they were not receiving enough water at the beginning of their life cycle. To improve our aquaponic system we could have placed another plastic box identical to the “tank” on top of the lip, to contain moisture and make mini green houses for the plants.

Many of the zebra fish died during the second week of the experiment; we tried to continue the experiment instead using feeder goldfish, most of which survived. We had no access to the fish on weekends, and could thus not feed them. The tanks were also located near windows and did not provide much insulation from the cold weather outside. We also believe that the fish may have needed more water than our tanks were able to hold. We could have improved our experiment by regulating the temperature of the lab more, using larger tanks to house the fish, and placing more bubblers to provide oxygen for the fish.

Although nothing can be determined from our experiment, besides that fast plants need a lot of water to germinate, aquaponics is a scientific field with a lot of potential. If our plants had germinated, and if we had been able to measure a large difference in height between the plants being fertilized by the fish being fed more nutritious food than

by the fish being fed less nutritious food, it could have been a solid starting point for farmers who desire new ways to make their crops larger without genetic modifications.

ACKNOWLEDMENTS

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Expression of Perforin and Granzyme B in the Liver and Spleen of BEP Neuron - and Cortical Cell - Implanted Rats Induced with Liver Cancer

By Rahul Rakhit'13, Dr. S. Murugan, Dr. Dipak Sarkar
Rutgers University Endocrine Facility

ABSTRACT

It is estimated that 1,500 people die each day due to cancer. Although the survival rate has increased to 66% from 51% in 1977, one out in of every four deaths is still attributed to cancer (7). Within the past few years, emerging evidence has shown that stress can impact cancer growth and metastasis by modulating nervous, endocrine, and immune systems (5).
The body's stress response is a release of corticotropin, by the corticotropin-releasing hormone (CRH). Beta-Endorphin (BEP) neuronal cell bodies are primarily localized in the arcuate nuclei and its terminal projections are distributed throughout the CNS (central nervous system), including PVN of the hypothalamus. The paraventricular nucleus (PVN) is a key nucleus that receives neuronal inputs from the other areas of the brain and other nuclei of the hypothalamus. In the PVN these neurons synapse with CRH neurons and inhibit CRH release during stress (2).
BEP is an endogenous opioid polypeptide, and it is a cleavage product of the POMC precursor peptide. Proopiomelanocortin (POMC) stems from autonomic neurons in the brain stem and spinal cord, thereby modulateregulate the ANS (autonomic nervous system). These types of neurons are called Hypothalamic Projection Neurons (6).
BEP neurons in the hypothalamus are able to control the growth and progression of tumor cells by modulating the neurotransmission in the autonomic nervous system and activating immune cell functions. BEP neurons stimulate the parasympathetic nervous system, which then releases acetylcholine (Ach) and and also causes suppression of the sympathetic nervous system and release of norepinephrine (NE). This chain of events leads to an activation of innate immune cells, including macrophages and natural killer cells (NK cells) , and an increase in cytotoxic immune cells and anti-inflammatory cytokine levels in circulation (1). In a tumor microenvironment, these immune cell and cytokine changes increase apoptotic death of tumor cells and reduce inflammation-mediated epithelial-mesenchymal transition (EMT), and thereby suppress cancer growth and progression. Collectively, these effects create an unfavorable environment for tumor initiation, growth and progression (3).

INTRODUCTION

I was focused on the protein, Perforin, and the enzyme, Granzyme-B. Perforin is a protein in the human body that is encoded by the PFR1 gene. It is a cytolytic protein found in the granules of cytotoxic T lymphocytes and natural killer cells. Perforin is used by natural killer cells and cytotoxic T lymphocytes to attack tumor cells. Granzyme B is an enzyme that cleaves peptide bonds in proteins, and is expressed

by cytotoxic T lymphocytes and natural killer cells (4). When these proteins are released from natural killers in close proximity to a targeted cell, perforin forms pores in the cell plasma membrane of the target cell and creates an aqueous channel through which the granzymes and associated molecules can enter and induce either apoptosis or osmotic cell lysis (5). My belief was that, in both the liver and the spleen, treating them with BEP would show a greater concentration of cytotoxic proteins, which in turn would create an unfavorable environment for tumorous growth. My objective was to prove whether BEP neuron implantation increases the levels of perforin and Granzyme B in the liver and spleen of liver cancer- induced rats.

MATERIALS AND METHODS

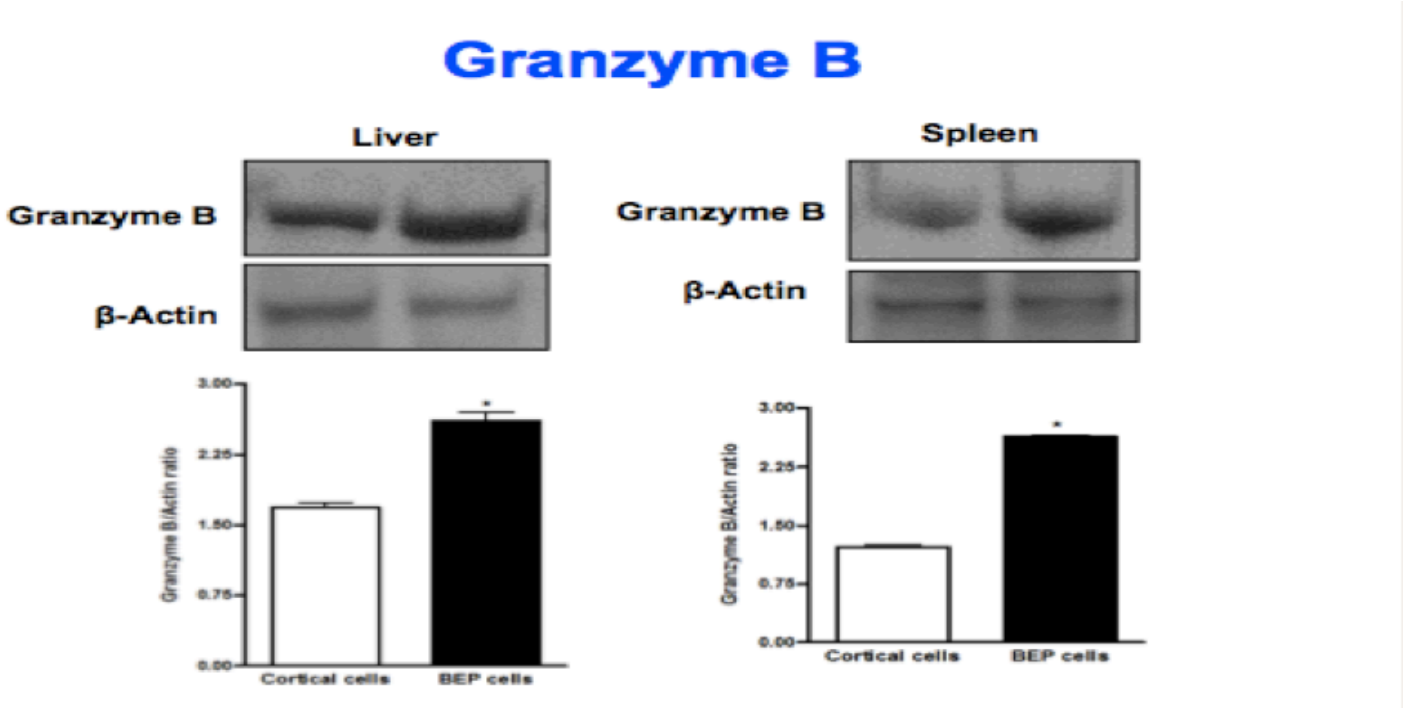
First, a set of ten, 50 µg samples were carefully extracted from liver and spleen proteins of sacrificed rats [Adult Fisher CDF rats (Charles River, MA) (120-150g)], (Rats were fed alcohol at increasing amounts, offspring were induced with liver cancer and to be used as subjects) and were then separated by a series of 4% to 20% SDS-PAGE. Afterward, the samples were transferred over night to immobilon-P polyvinylidene difluoride (PVDF) membranes. After 12 hours, the membranes were incubated with a primary antibody (Perforin-rabbit polyclonal and Granzyme B-mouse monoclonal, Santa Cruz Biotechnology Inc., Santa Cruz, CA; 1:100) overnight at 4°C in blocking buffer (5% nonfat dry milk in TBS). Membranes were then washed and incubated with peroxidase-conjugated secondary antibody (1: 5,000) for one hour. Finally, membranes were washed, and the signals were visualized by Pierce- enhanced chemiluminescence Western Blotting substrate (Thermo Fisher Scientific Inc., Rockford, IL). The membranes were exposed to X-ray films and developed using an X-Ray developer. β-Actin served as an internal control for loading and transferring of equal amounts of protein samples. The immunoblot bands were quantified through analyzing the optical densities of scanned bands using the NIH Image software.

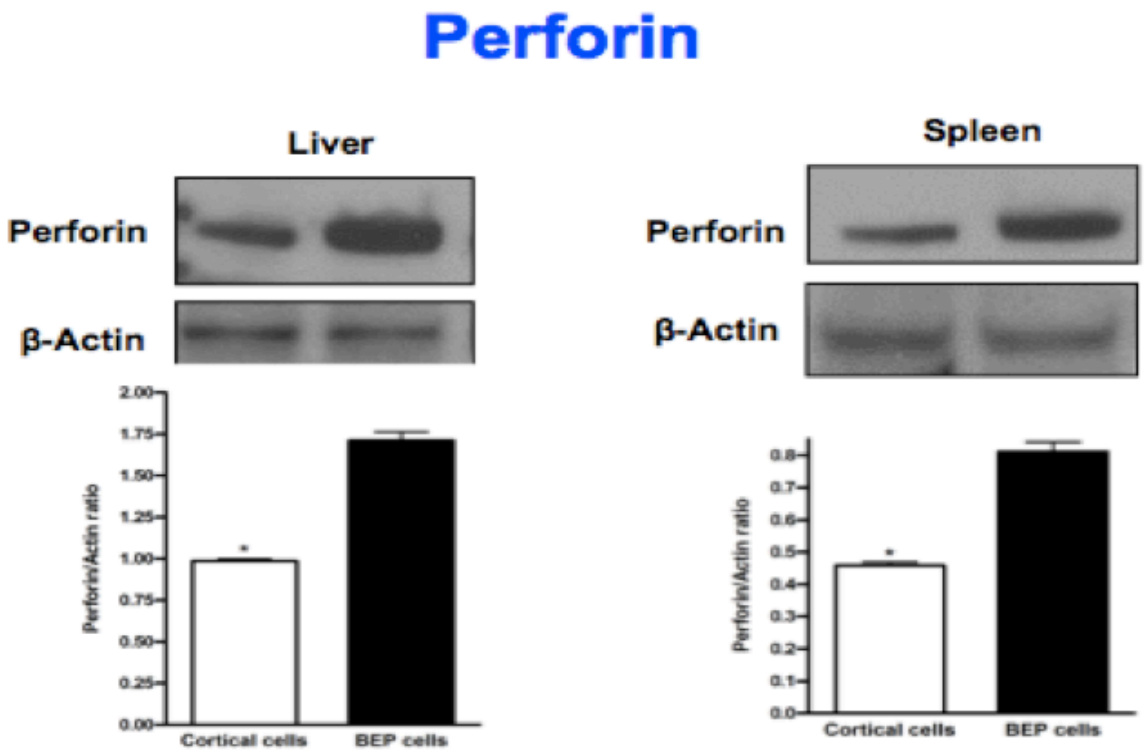
RESULTS

The present results shows that BEP neuron - implanted rats had higher levels of perforin (Figure 1) and Granzyme B (Figure 2) in the liver and spleen when compared to cortical cells implanted rats, which were induced for liver cancer. The levels of perforin and Granzyme B were higher in the liver than spleen in both groups, which confirms some previous research that the liver expresses more NK cell proteins and potent than the spleen. The anti-tumorigenic effects observed in this study could also be due to to the higher expression of NK cell proteins in BEP neuron - transplanted rats. The Beta-actin levels in both the liver and spleen of the implanted rats shows that the loading of the gel was done successfully and the results were not tampered with.

DISCUSSION

The raise in levels of Perforin and Gran-





zyme-B in BEP neuron implantations in the liver and spleen of liver cancer induced rats shows that increased levels of BEP neurons hold a key role in creating an anti-tumorigenic environment. Because of the changes in levels of immune cells and cytokines, the apoptotic death of tumor cells is increased and the inflammation-mediated epithelial-mesenchymal transition (EMT) is reduced, therefore suppressing cancerous tumor growth and progression. With this experiment, it seems clear that Perforin and Granzyme-B are a clear-cut answer to suppress cancer because of its large difference when compared to cortical cells, but further research needs to be done on these proteins. If there is a lack of Perforin in the body, it can lead to an upsurge in cancerous cell growth. Perforin can also be put to blame when the wrong cells are targeted for death, such as in autoimmune disease conditions, like early onset diabetes, or in tissue rejection following bone marrow transplantation. With more research, Perforin can hopefully be boosted to effectively protect against cancer and other harmful diseases.

CONCLUSION

In conclusion, the transplantation of BEP neurons in the liver and spleen of liver cancer- induced rats increases the levels of Perforin and Granzyme-B. By increasing the production of Perforin and Granzyme-B, which are found in cytotoxic-T lymphocytes and natural killer cells, an anti-tumorigenic micro-environment is created in the organs to equip it to suppress cancer growth and progression. While this research focused primarily on effects on the liver and spleen affected by liver cancer, in the future more research will be done to test the BEP neuronal effects in multiple other areas regions of the body.

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Correlating Incline and Heart Rate in Teenage Male Runners

By Sam Ricciardi'13

ABSTRACT

The goal of this experiment was to determine a precise relationship between heart rate increase and incline increase in teenage male runners. Using a heart rate monitor, we recorded the heart rates of five teenage males every 30 seconds at four different inclines with the pace held steady over the course of several runs. Their maximum and resting heart rates were then determined. These values were averaged, converted to a percentage of maximum heart rate and graphed together. Unfortunately, due to systematic errors within the experiment, a universal relationship between heart rate and incline could not be established. Despite this, some interesting observations were made.

INTRODUCTION

The goal of this experiment was to determine the exact relationship between incline and heart rate in teenage male runners. Five teenage male runners with varying experience ran 1 mile at an 8:34 pace four times. For each run, the incline was changed (0, 5.0, 10.0, 15.0). Their heart rates were recorded every thirty seconds during each run. Their heart rate during the run was then compared to their maximum and resting heart rates in order to determine relative "difficulty." The goal of this experiment was to find a universal relationship mapping increase in heart rate to increase of incline. Incline training can enhance training efficiency (1). Heart rate training can also increase efficiency. Therefore, finding a universal relationship between heart rate and incline could allow for general training at maximum efficiency.

MATERIALS AND METHODS

Five teenage males with varying running experience were chosen. A Polar RS300X heart rate watch was used with a Polar WearLink heart rate monitor to determine their heart rate. The WearLink has two electrodes on its interior side that were doused with water before each run. The WearLink was strapped tightly just below each participant's nipples. The runners were then brought up to the Pingry gym where they ran on the right-most treadmill four times each.

For each run, the incline was set beforehand and held constant. The start button was then hit on the RS300X watch, and the runner's heart rate was displayed. The pace was then set to 3.0 for 1 minute. No heart rate was recorded during this "warm-up." After 1 minute, the pace was raised to 7.0 (8:34 minutes/mile). Each runner ran at this pace for one mile or until failure.

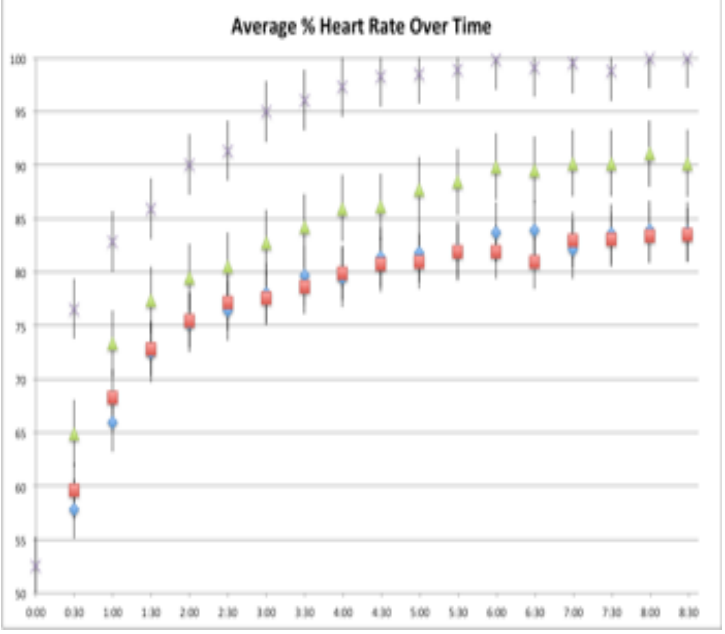
The inclines used were 0.0, 5.0, 10.0, and 15.0. Each incline was used once per runner. Due to hectic Pingry schedules, runners were not brought in consistently. Although some ran four days straight, others had weeks-long gaps in between their runs.

Each runner's maximum and resting heart rate was found. Each max heart rate was found by taking the runner's highest heart rate at the 15.0 incline. Each resting heart rate was found by taking the runner's pulse for a minute after they had been sitting for a few minutes.

Each runner's heart rate was recorded in an Excel spreadsheet every thirty seconds during each run. Using each max and resting heart rate, each heart rate data point was converted into a percentage of maximum heart rate. These values were then averaged and graphed with standard error bars.

RESULTS

Fig 1. Blue indicates 0.0 incline.
Red indicates 5.0 incline.
Green indicates 10.0 incline.
Purple indicates 15.0 incline.



DISCUSSION

The goal of this experiment was to be able to accurately predict a runner's heart rate (at an 8:34 pace) at any given time for any given incline. Due to the many errors of this experiment, it is impossible to accurately predict a runner's heart rate at any given time for any given incline. To enumerate these errors:

Only five runners were used.
Only four inclines were used.
Their runs were sporadic and spread out; they did not follow a set schedule.
They were doing additional winter track or other workouts that may have tampered with their heart rates.
Their max heart rates were determined using the 100% incline run as opposed to a separate sprint.
Their resting heart rates were not determined after a long period of rest, were not calculated with the RS300X, and were only calculated once.
Some runners did not complete the 100% incline run.

If this experiment were to be done again, it should be done in the following manner:

More runners should be used.
They should follow a set schedule and not do any other workouts.
Their resting and max heart rates should be determined before and after all runs were completed. These should be averaged.
The inclines used should be 0, 5.0, 10.0, 12.7, 14.3 15.0.
The runners should not run for a set distance. They should run until they reach their max heart rate.
The graphs should compare the time it took to reach their max heart rates.

Despite these technical errors, some interesting observations can be made from the data.
Running an 8:30 mile at a 5.0 incline produces very similar results to a 0.0 incline.
Despite having runners with extremely varied experience, there was a consistent trend for each incline.
0.0 and 5.0 inclines appear to flat-line around 83%.
10.0 incline appears to flat-line around 90%.

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Using Algae to Create Biodiesel

By Mikell Graves'13, Cole McCollum'13, and Harrison Homer'13

ABSTRACT

An effort was made to create biodiesel fuel from endogenous sources on the Pingry Campus. Algae from the local pond were grown in bioreactors. Lipids were extracted from algae and trans-esterified to create biodiesel fuel. In the experiment, the algae were successfully grown; the Folch method was used for lipid extraction, and the product trans-esterified. No biodiesel, or no quality biodiesel, was recovered using our techniques.

INTRODUCTION

The purpose of this project was to develop a method for producing biodiesel on a small scale. Diesel fuel is a complex mixture of hydrocarbons with a boiling range between 350 and 650 degrees Fahrenheit, used in diesel engines and ignited by compression. Biodiesel is a form of diesel made from renewable plant-based sources. It has the advantage that it is also non-toxic and biodegradable. Biodiesel is an alkyl-ester typically derived from reacting lipids with alcohol producing fatty-acid esters. Algae have been shown to be significantly more efficient per acre than any other crop in producing biodiesel. Algae are a viable option for small-scale use in a home, laboratory, or school setting. The creation of sustainable, "home-grown" oil is beneficial ecologically, economically, and politically. It is one of a variety of alternative fuels that can run in a standard diesel engine. We designed a closed-system photo-bioreactor to cultivate algae. Algae oil, the lipids stored in the algae cells, can be isolated and turned into biodiesel through a process known as trans-esterification. The goal of this project was to demonstrate the feasibility of accomplishing this, with the ultimate goal of achieving sufficient quantity and quality for practical application to serve the campus's needs.

MATERIALS AND METHODS

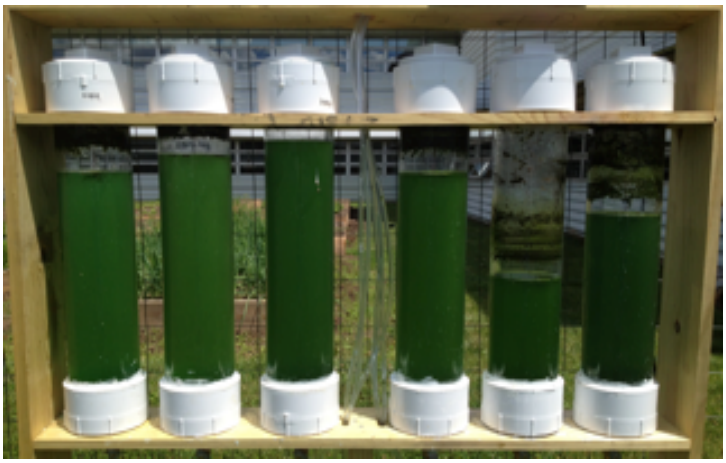
In order to create biodiesel, the oil source needed to be obtained, which in our case was algae. The oil then needed to be isolated, and then finally trans-esterified in order to create the fuel.

Algae were obtained from ponds on the Pingry campus. Pond water was used as a medium and Miracle-Gro was used as a growth accelerant. The algae were grown in a bioreactor based on modular algae PhotoReactor V.2 from a design from Inventgeek.com, and modified to fit local circumstances. In accordance with the design, the water in each tube was constantly agitated by a stream of air in order to assure a continuous supply of oxygen and carbon dioxide. After two weeks, the algae were harvested. The tubes containing water and algae were placed through a filter. The remaining material underwent flocculation. The flocculate was then centrifuged and the pellets were removed. An alternative method of algae harvesting was carried out to try and enhance the yield. This involved evaporation and drying of the algae in Petri dishes in the incubator. The algae were then removed from the plates and the lipids extracted.

Lipids were extracted from the pellets through the Folch method. This method involves mixing 1.67 mL of methanol and 3.34 mL of chloroform, in keeping with the 1:2 ratio needed. This total mixture of 5 mL was added to .5 mL of algae (though the mixture to algae ratio should have been 20:1). Together, the new mixture was shaken at room temperature for 17 minutes to assure thorough mixing. The solution was then centrifuged at 2,000 rpm for 10 minutes. 1.1 mL of a 9% salt solution was added to the result of centrifugation (.9% is called for in the method).

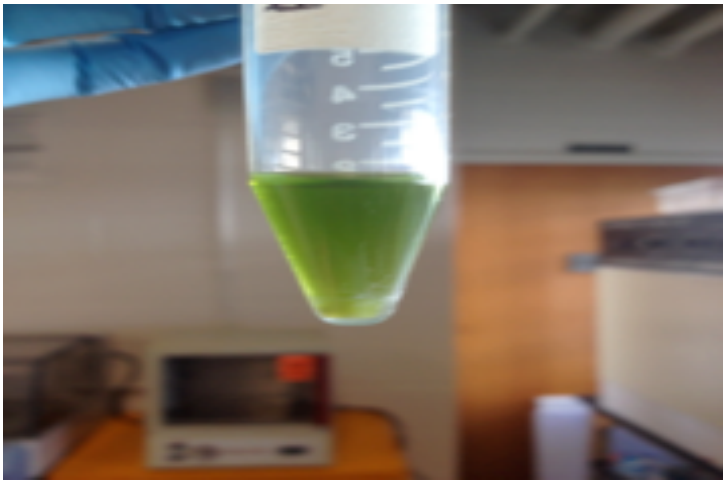
Trans-esterification is accomplished by creating a strong base and mixing it with the previously extracted lipids. In this case, the strong base was sodium methoxide. This was synthesized by thoroughly mixing sodium hydroxide with methanol. The resulting mixture was then added to the lipid extract and blended. This was then left to settle. After settling, two layers were identified. The supernatant was biodiesel (an alkyl-ester), which was then decanted. The remaining material was glycerin, which was a byproduct.

Fig. 1: Algae growth in photo-bioreactor



The Wash Test and Methanol Test were carried out to determine the purity of the product. In the Wash Test, equal parts of water and biodiesel are added to a jar, shaken violently for 10 seconds, and allowed to sit for 30 minutes. If they do not separate in 30 minutes or less, it is defined either as no diesel or as poor quality diesel. The Methanol Test involves mixing a 1:9 ratio of diesel to methanol. After 30 minutes, the solution should be clear with nothing settled at the bottom.

Fig. 2: Results from testing. Green color represents poor quality diesel or no diesel at all.

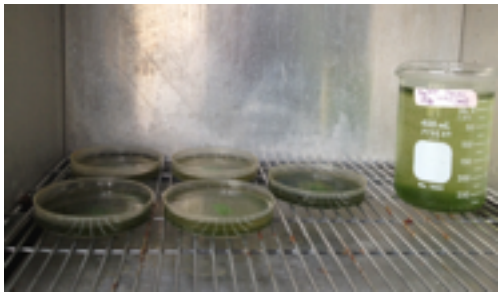


RESULTS

We were not able to obtain any diesel. The Wash Test and Methanol Test were negative, indicating no diesel or poor quality diesel.

Fig. 3: Light yellow color of results after trans-esterification suggests poor quality diesel

Fig. 4: Algae drying in incubator prior to trans-esterification.



DISCUSSION

A variety of factors may have led to the failure to obtain any quality diesel. The error could well have occurred during lipid extraction, when non-standard ratio of algae to chloroform-methanol mix was used. Another error may have involved the use of excessively high concentrations of salt solution, which may have disrupted the lipids. There appeared to be no step designed to quantify the amount of lipid extracted from the algae prior to attempting trans-esterification. It is also possible that the algae were not mechanically broken down sufficiently to allow access to the lipids. Upon reviewing other protocols, this is an essential step that requires more than simple centrifugation and a short vortex. It may require more intensive mechanical disruption of cell walls, including blending and lyophilization.

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