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LETTER FROM THE EDITOR

Since its founding in 2015, *Perpetua* has fostered the development of talented undergraduates, working with faculty mentors to create a new platform for students to share their research. The journal is proud to support students in all fields of study and promote mentor-supported research taking place at the University of Alabama in Huntsville.

Perpetua is Latin for "unceasing." This name was chosen to represent the unceasing pursuit of knowledge at the heart of academia. Our goal is to recognize the tireless efforts of undergraduate scholars at UAH by providing the opportunity for publication and scholarly review of their research. *Perpetua* is undergraduate-run, which provides students who work on the journal hands-on experience with scholarly publishing and the peer-review process.

In the future, we hope to expand our reach to share the interdisciplinary research taking place at UAH with the broader academic community. We aspire to connect undergraduates with new opportunities in research, and inspire students in their pursuit of knowledge.

Kelsey McKee Editor-in-chief *Perpetua*

SPECIAL THANKS

Perpetua would like to thank our faculty advisors for their guidance and advice, without which the journal would not be possible. The faculty advisors' vision to create a journal specifically for undergraduates has given the students at UAH new opportunities to receive support and mentorship. We would like to give a special thanks to Dr. Gordon MacGregor for offering his input and advising the staff on how to improve the journal.

Special thanks to the graduate students who served as reviewers for their willingness to provide their expertise and support the research being performed by undergraduate students. We also thank the faculty mentors who encouraged their undergraduate researchers to submit their manuscripts for publication.

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Cytotoxicity of Plant Extracts on MCF7 and Hs578T Breast Cancer Cell Lines

Brianne Brazell Department of Biological Science

Abstract – The cytotoxicity of various essential oils from plant extracts has been tested on two different breast cancer cell subtypes. An estrogen positive breast cancer, MCF7, and a triple negative breast cancer, Hs578T, were both used in this study. Concentrations (weight volume w/v) of test samples of 0.01%, 0.005%, 0.004%, 0.003% were used to determine the LC₅₀ value for compounds determined to decrease the number of viable cells in a culture. Testing completed with MCF7 cells determined that the fraction 2C of Terminalia catappa (Teca) was the most efficient for cytotoxicity, with a percent kill of 80.56 at a concentration of 0.004% (w/v). Hs578t testing determined that fraction 3 of Ipomoea tricolor (IT) had the most efficient toxic effects, with a percent kill of 93.53 at a concentration of 0.003%(w/v), which was the lowest concentration tested.

I. Introduction

In the United States, one in every eight women is expected to be affected by breast cancer in her lifetime [1]. The problem with treating breast cancer is that it is not just one disease, and each form of the disease responds to treatments differently. Current chemotherapy protocols have several limitations, including the fact that these treatments do not only damage cancer cells but also the healthy cells. Additionally, cancer cells can develop mutations in their DNA, which allows them to become resistant to chemotherapeutic agents.

Breast cancer can be divided into different clinical subtypes. These subtypes are based on tests for three molecular markers: expression of estrogen receptor (ER), expression of progesterone receptor (PR), and amplification of HER-2/Neu. Estrogen receptor positive cells have receptors for estrogen, suggesting that their growth is promoted by signals it receives from estrogen [2]. HER2-positive cell types produce too much of the protein HER2, a mutated EGF receptor, making the cancer aggressive and fast growing [3]. Breast cancer cells that test negative for all three of the molecular markers are classified as triple negative. Triple negative cell types tend to have a less favorable prognosis than the hormone receptor expressing cell types [4]. Hormone receptor positive cell types are present in about two out of every three breast cancer diagnoses [2]. Triple-negative cell types occur at a much lower rate of about 10-15% [4].

In this study, we tested an estrogen-positive breast cancer cell type, MCF7, and a triple-negative breast cancer cell type, Hs578T. These cells were used in the cytotoxicity testing of various essential oils from plant extracts. Cytotoxicity testing is an important step in determining the effects of compounds on cell viability. In standard cytotoxicity testing methods, cell monolayers are grown to near confluence and then exposed to test samples directly by adding the compounds to the culture media. Cells may be observed for visible signs of toxicity, such as a change in size or appearance of cellular components [5]. Assays may be performed to determine the viability of the cells as well. One such assay is called a MTT assay, which is based on the selective ability of viable cells to reduce 3-(4,5-dimethylthiazol-2-yl)-2,5diphenyl tetrazolium bromide into purple formazan crystals. The formation of these crystals is reliant on functioning metabolic activity, which makes it a useful method for testing for cytotoxicity [6]. The data collected from the MTT assay can be further analyzed to find the LC_{50} value for the compounds. This test is used to determine the lethal concentration of a compound required to kill fifty percent of the cells in a culture.

II. Materials and Methods

Removing Cells from Cryopreservation

The cryovial containing cells was removed from liquid nitrogen and immediately immersed in a 37°C water bath. The cells were quickly thawed by swirling in the bath. Then, the cells were transferred to a centrifuge tube and suspended in RPMI 1640 growth media supplemented with 10% fetal bovine serum. The cell suspension was then centrifuged for three to four minutes, and the supernatant was removed using suction, being careful to not disturb the pellet. The cells were gently suspended in the growth medium once more and divided evenly between two T-25 flasks.

Cell Culture

Cells were maintained in a T-25 culture flask with RPMI 1640 growth media with 10% fetal bovine serum at a plating concentration of 2.5×10^5 cells/mL per flask and 5 mL/ flask. The cells were incubated at 37°C and 5% CO₂ in a humidified chamber. The cells were grown in RPMI 1640 media with L-glutamine, 26 mL of 7.5% sodium bicarbonate per liter medium, 10,000 units of penicillin and 10,000 µg/mL streptomycin per liter of medium, and 15 mL of 1M HEPES per liter medium, buffered with 5.0 N NaOH to pH 7.35. The media was then sterile filtered using a Corning[®] bottle-top filter into two 500 mL bottles. The final growth media contained 10% fetal bovine serum. The growth media was exchanged every 48 hours, and cells were passaged once a week. Generally, two flasks of cells were maintained at a time.

Passaging Cells

Cells were removed from the flask using 1X Hank's Balanced Salts Solution, without calcium or magnesium, containing 5.0 g porcine trypsin, 2 g EDTA-4 Na per liter in 0.9% NaCl. The growth media was removed with suction, and the cells were washed with 5 mL of 1X Hank's Balanced Salts Solution. The Hank's solution was removed, and 5 mL of the trypsin-EDTA/Hank's solution mixture was added to the cells. After one to two minutes, some of the trypsin-EDTA/Hank's solution was removed so that the remaining liquid just covered the surface of the cells. To assist the cells' separation from the surface, the flasks could be placed in the CO₂ incubator for one to two minutes. After the cells could visibly be seen releasing from the surface, 5 mL of growth media was added to the flask. Any remaining cells on the flask surface were washed off with the growth media, and the contents of the flask were transferred to a centrifuge tube. The cells were then centrifuged for two to three minutes. The supernatant was removed using suction, being careful to not disturb the pellet. Then, the pellet was suspended in growth media. After the pellet was suspended, 0.1 mL of cells was added to a solution of 0.3 mL of 0.4% Trypan Blue Stain and 0.6 mL of 0.15 M NaCl. The cells were then counted using a hemocytometer. The appropriate number of cells were then distributed to the desired culture vessels.

MTT Assay

Cells were plated at a concentration of 1.44x10⁶ cells per well in a 96-well plate in a volume of 0.1 mL. Test samples were diluted in growth media to a concentration of 0.01% (w/v). Tingenone was used as a positive control, and it was prepared like the other test samples. The growth media and 0.01% (w/v) DMSO were used as negative controls, as the test samples were dissolved in DMSO. The media was removed from the cells using suction, and 100 µL of the test sample dilutions were added to the designated wells. The cells were incubated in these test samples at 37°C and 5% CO₂ for 48 hours. On the day of the assay, a 1:10 dilution of 5mg/mL of stock MTT in growth medium was made to give a final concentration of 0.5 mg/mL. The media on the cells was then removed using suction, and 100 µL of the MTT solution was added to each well. A 96 well plate SpectraMax spectrophotometer was used to immediately read the absorbance at 570 nm. The plate was then placed back in the incubator for three to four hours at 37°C and 5% CO₂. After the incubation period, the media was carefully aspirated from the wells, being careful not to disturb the purple formazan crystals. Then, 100 µL of ISO PBS, containing 100 mL isopropyl alcohol, 4.0 µL 5.0 N HCl, and 50.0 mL phosphate-buffered saline, was added to the wells, and the plate was gently shaken to dissolve the crystals. The post read was then completed on the spectrophotometer at 570 nm. This data was compiled in Microsoft Excel to calculate the percent of cells killed, the percent of viable cells, and the standard deviation for each.

LC50 Analysis

Compounds with a high percent of killed cells and a low standard deviation were chosen for the LC_{50} analysis. Concentrations of 0.01% (w/v), 0.005% (w/v), 0.004% (w/v), and 0.003% (w/v) were tested for each of the chosen samples. The same procedure for the MTT assay was followed.

III. Results

Table 1: MCF7 MTT Assay Results

Extract/ Sample	% Viable	% Kill	Std. Dev. of % Kill
ABBAEO	13.10	86.90	7.95
BOCAEO	-0.99	100.99	1.56
CAODEO	0.65	99.35	5.29
CILAEO	-1.45	101.45	1.54
COOFEO	4.78	95.22	2.75
CUSEEO	10.86	89.14	12.82
LAANEO	39.79	60.21	17.53
MYCOEO	66.08	33.92	13.48
SAAUEB	2.07	97.93	0.45
Teca 2C	2.13	97.87	1.24
Teca 3B	5.34	94.66	1.82

Table 1 shows the resulting data from MTT assays completed with MCF 7 cells with test samples at 0.01% (w/v). The percent of viable cells, percent of killed cells, and the standard deviation of the percent of killed cells are shown. Samples ending in "EO" are essential oils. Data from this assay was used to determine which Samples would be used for further analysis.

Conc. of Std. Extract/ % Sample % Kill Dev. of Sample Viable (w/v)% Kill Teca 0.01% -4.16 104.16 3.76 2CTeca 0.005% -1.13 101.13 2.76 2C Teca 0.004% 80.56 19.44 6.83 2C Teca 0.003% 58.42 41.58 8.30 2CTeca 0.01% -2.85 102.85 3.23 3B Teca 0.005% 36.67 63.33 3.50 3B Teca 0.004% 83.21 16.79 5.21 3B Teca 0.003% 50.76 49.24 10.96 3B Table 2 shows the results from the LC_{50}

Table 2: LC₅₀ Analysis of MCF7 Cells

Table 2 shows the results from the LC_{50} analysis with MCF7 and Teca 2C and Teca 3B test samples. The concentration of the sample, percent viable cells, percent killed cells, and the standard deviation of the percent of killed cells are shown.

Extract/ Sample	% Viable	% Kill	Std. Dev. of % Kill
ABBAEO	8.38	91.62	4.84
BOCAEO	6.21	93.79	6.95
CAODEO	4.17	95.83	2.52
CILAEO	-5.03	105.03	12.34
COOFEO	6.14	93.86	9.81
CUSEEO	2.98	97.04	1.74
LAANEO	5.45	94.55	1.49
MYCOEO	5.54	94.46	3.55
SAAUEB	4.30	95.70	1.19
IT 1	83.10	16.90	41.64
IT 2	1.03	98.97	1.73
IT 3	1.58	98.42	0.55
IT 4	1.30	98.70	2.68
IT 5	45.83	54.17	21.29
IT 6	21.81	78.19	8.81
IT 7	1.50	98.50	0.68
IT 8	15.94	84.06	6.25
IT 9	7.06	92.94	2.48
IT 10	19.63	80.37	3.65

Table 3 shows the results of the MTT assay

on HS 578 T cells with various test samples at 0.01% (w/v). The resulting percent of viable cells, percent of killed cells, and the standard deviation of the percent

of killed cells are shown.

Table 3: Hs578T MTT Assay Results

Table 4: LC50 Analysis of Hs578 T Cells

Extract/ Sample	Conc. of Sample (w/v)	% Viable	% Kill	Std. Dev of % Kill
CAODEO	0.01%	3.68	96.32	2.11
CAODEO	0.005%	60.64	39.36	26.72
CAODEO	0.004%	58.99	41.01	22.83
CAODEO	0.003%	86.08	13.92	38.58
CILAEO	0.01%	7.16	92.84	6.37
CILAEO	0.005%	54.10	45.90	10.45
CILAEO	0.004%	58.06	41.94	3.78
CILAEO	0.003%	56.22	43.78	11.37
IT 2	0.001%	4.64	95.36	5.31
IT 2	0.005%	28.86	71.14	13.20
IT 2	0.004%	35.44	64.56	4.16
IT 2	0.003%	54.22	45.78	18.69
IT 3	0.01%	-1.06	101.06	0.48
IT 3	0.005%	-0.74	100.74	0.81
IT 3	0.004%	-3.44	103.44	6.59
IT 3	0.003%	6.47	93.53	3.52
IT 4	0.01%	4.43	95.57	3.39
IT 4	0.005%	34.58	65.42	20.76
IT 4	0.004%	30.03	69.97	14.15
IT 4	0.003%	61.99	38.01	20.78

Table 4 shows the LC_{50} analysis of Hs578 T cells with different test samples. The concentration of the test sample, the percent of viable cells, the percent of killed cells, and the standard deviation of the percent of killed cells are shown.

III. Discussion

Many compounds were tested on both the MCF7 and Hs578T cells. Some of the samples had about equal kill percentages, while others had different outcomes in the two cell types. For example, MYCOEO killed 94.46% of the Hs578T cells (Table 3), while it only killed 33.92% of MCF7 cells (Table 1). The sample LAANEO had a similar effect without as drastic of a difference, killing 94.55% of Hs578T cells (Table 3) and 60.21% of MCF7 cells (Table 1).

The MTT assay was performed using test samples at a 0.01% (w/v) concentration. Growth media and 0.01% (w/v) DMSO were used as negative controls, while 0.01% (w/v) Tingenone was used as a positive control for cytotoxicity. The data obtained from the MTT assay was used to decide which samples were used in further analysis. Samples with a percent kill of above 90% with a standard deviation less than 15 were used to complete an LC₅₀ analysis. For MCF7 cells, the samples chosen were Teca 2C and Teca 3B. Teca 2C had a percent kill of 97.87% and a standard deviation of 1.24, while Teca 3B had a percent kill of 94.66% and a standard deviation of 1.82 (Table 1). For Hs578T cells, the samples chosen for the LC_{50} were CAODEO, CILAEO, IT2, IT3, and IT 4. The sample CAODEO had a percent kill of 95.83% with a standard deviation of 2.52, CILAEO had a percent kill of 105.03% with a standard deviation of 12.34, IT2 had a percent kill of 98.97% with a standard deviation of 1.73, IT3 had a percent kill of 98.42% with a standard deviation of 0.55, and IT 4 had a percent kill of 98.70% with a standard deviation of 2.68 (Table 3).

The LC₅₀ analysis was performed to determine the lowest concentration at which the sample would still kill 50% of the cells tested. For MCF7 cells, Teca 2C could be used at 0.004% (w/v) to kill 80.56% of the cells, but at 0.003% (w/v) it only killed 41.58%. Teca 3B had a percent kill of 63.33% at a concentration of 0.005% (w/v). At 0.004% (w/v) Teca 3B, only 16.79% of cells were killed. The standard deviations for all concentrations tested were relatively low, which indicates that the results are accurate. For Hs578T cells, CAODEO had a percent kill of 96.32% at a concentration of 0.01% (w/v), but at a concentration of 0.005% (w/v) only 39.36% of cells were killed. For CILAEO, 92.84% of cells were killed at the 0.01% (w/v) concentration, but only 45.90% of cells were killed at the 0.005% (w/v) concentration. The compound IT2 had a percent kill of 64.56% at a concentration of 0.004% (w/v), and the percent kill at a concentration of 0.003% (w/v) was 45.78%. The compound IT3 had a percent kill above 90% at the lowest concentration tested of 0.003% (w/v). IT4 had a percent kill of 69.97% at a concentration of 0.004% (w/v), and it had a percent kill of 38.01% at 0.003% (w/v).

Overall, the fraction Teca 2C had the best cytotoxicity results for MCF7 cells, while IT3 had the best results for Hs578T cells. Going forward, the samples of IT1-IT10 should be tested on MCF7 cells, while the fractions Teca 2C and Teca 3B should be tested on Hs578T cells. Additionally, an LC₅₀ should be performed with BOCAEO and CILAEO on MCF7 cells. The LC₅₀ for the Hs578T cells should be repeated based on the results of the most recent MTT assay. The Hs578T MTT assay was repeated due to high standard deviations, and a new LC₅₀ analysis should be performed to coincide with these results. All samples will be further fractionated to isolate new cancer drugs.

IV. Ackowledgments

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Mallard Fox Creek Industrial Park and Cummings Research Park: Investigating Employment and Income Impacts

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Abstract – A research park and an industrial park are each a hub of economic activity and their effect on the local economy can be measured in terms of jobs, income and output generated from its development. These sites provide an opportunity for the surrounding institutions and businesses to likewise develop post hoc. An economic impact study can shed light on the supply chain of the industries in the region, the kinds of jobs created, and the value of the industries in these parks to the region. The purpose of this paper is to investigate the economic impact of the Mallard Fox Creek Industrial Park in Decatur, Alabama, and Cummings Research Park in Huntsville, Alabama, and to understand the similarities and differences between the two. Interviews with key stakeholders, administrators, and participants within the parks were conducted in order to obtain qualitative and quantitative data on each park. Our findings suggest that the two parks are comparable in terms of job creation and income generation. Both parks observe significantly large job multipliers in steel/metals industries, and the research park observes significantly large income multipliers in research and development, management, and computer/engineering services at the state level. Disparities in the multipliers of each park increase when comparing local impacts to state impacts.

I. Introduction

Wassily Leontief, a Harvard economist, utilized data collected by the U.S. government to aggregate transactions of American individuals and firms on one "two-way table" (Leontief, 1936). His conceptual table could be modified by joining multiple accounts, i.e. rows or columns, to reflect transactions that occur between industries. This paper established the application of the theory that permits economic analyses such as the one exhibited in this paper.

Later, in 1967, Leontief elaborated upon his earlier findings by adjusting the model to reflect the intricacies of industrial interdependence. In doing so, Leontief was able to demonstrate the effects of one industry on another based upon the required inputs such as labor, capital, and raw materials. As an illustration, Leontief stated that "...the production of the nonmetal inputs absorbed by metalworking industries often requires the use of various metal products in its turn..." (Leontief 1967). This observation may be illustrated with the modified model through the utilization of matrix operations. Leontief's dedication to the model resulted in his Nobel Prize in Economic Sciences "for the development of the input-output method and for its application to important economic problems" (Nobel Media AB).

The idea of the input-output model is not universally accepted. Citing "unrealistic" assumptions about the output of firms within the model, some sought to loosen restrictions through modified definitions to create "a more realistic interpretation" of the model (Klein, 1952). Others, such as Carl F. Christ, noted that assumptions on singular inputs in industries had been abandoned and criticized Leontief's claims that the model is "a generalequilibrium system" because the model lacked information on preferences or demand for output (Christ, 1955). Similar to the Kingman Airport Industrial Park study, the authors will investigate the respective impacts of Mallard-Fox Creek Industrial Park and Cummings Research Park on their economic regions.

Today, various entities, including state governments (EDRG, 2012), industrial trade organizations (Dunham & Associates, 2015), industrial parks (Coffman, 2005), and universities (Mahalingam & Thompson, 2015, 2016) conduct or otherwise commission economic impact studies based upon the foundations that Leontief provided.

The application of Leontief's model is not limited to industrial parks. Other applications include investigating the environmental impact of energy production in the U.K. (Hawdon & Pearson, 1995) and general improvements, replacements, or upgrades to existing industries (Bess & Ambargis, n.d.). Each considers the direct, indirect, and induced effects of their respective subjects. The direct effects are employment and expenditures generated by the industry activity itself, while the indirect is the same for supply chain or suppliers of inputs, and induced, for industries affected by the spending of those employed in the industry.

Economic impact studies are undertaken to understand the effect that a particular industry or industries may have on the chosen region, either a city, county, multiple counties, or even the state. The spending or employment that occurs in that industry has an effect on the spending and employment in the entire region in which it is located, as discussed before. Generally, the indirect and induced effects are combined and compared to the direct effect to derive a multiplier for the industrial activity. For instance, Arizona State University and Coffman Associates found that the Kingman Airport Industrial Park's impact created over two and a half jobs for every job in the industrial park and observed a significant portion of indirect output resulting from the manufacturing firms in the park (Coffman).

This research undertakes to study the economic impact of two parks, the Mallard Fox Creek Industrial Park and Cummings Research Park, in an effort to quantify the contribution of these parks to the local economy. Mallard Fox Creek Industrial Park (MFCIP) is located in Decatur, Alabama, on the banks of the Tennessee River. The MFCIP covers over 1,000 acres of land in the city of Decatur. A Tennessee Valley Authority (TVA) dock, railroad lines, and tractor trailers comprise the supply chain of the fifteen firms located there. Most firms are steelworking firms, but some chemical processing and rocketry is also based in the park (MCEDA).

Cummings Research Park (CRP) is located in the middle of Huntsville, Alabama, directly adjacent to the University of Alabama in Huntsville and the Redstone Arsenal Missile Defense Base. The result of collaboration between Milton Cummings and Wernher von Braun, the CRP was founded shortly after 1962 as an effort to promote a high-tech university-industry partnership to support the young NASA program at the height of the space race. Since then, the park has grown, with research and development driving much of its expansion. CRP hosts over one hundred firms, several of which are Fortune 500/100 companies. Because of its surroundings, the CRP hosts several engineering-related firms as well as auxiliary services for the firms. To this day, the CRP attracts several contractors partnered with the Arsenal and NASA, both of which have maintained their presence in Huntsville (Chamber of Commerce).

Both parks have had significant impact on the regions that they are located in with regards to

economic development. While both the industrial and research park have developed supply chain industries to support them, both parks have also developed wholesale trade, retail sales, and other industries where the parks' employees spend their incomes. Even so, it is possible to see some differences in the types of industries developed by each of these parks. While the MFCIP has manufacturing and steel firms, the CRP has communication, electronic, computer, and engineering services as the primary industries.

The rest of the paper is organized as follows: Section 2 describes the methodology that describes the data collection and processes that were used in the study to derive the results. Section 3 presents and discusses the results, and Section 4 presents the conclusion where the overall results are discussed.

II. Methodology

In order to use the Leontief input-output model effectively, a list of firms currently operating in the MFCIP and CRP was gathered after interviews with the Chamber of Commerce of Huntsville. Madison County, and respective directors of both the organizations. The industrial directory for the industrial park was used to determine the relevant employment data and industrial codes along with interviews and surveys of relevant stakeholders. The directory for the Cummings Research Park was available on the park's website (Chamber of Commerce). Interviews with the director of Cummings Research Park, and the Chamber of Commerce of the city of Huntsville provided data for a number of firms as well. There were a few other firms listed but many attempts and searches for data did not yield any response. Most firms are included in this study; however, the firms with no data were excluded. The study was conducted for the year 2015.

One of the challenges in measuring the economic impact of a research or industrial park is accounting for leakages, individuals who work in one geographic area but live and spend their earnings in a geographic region outside of the focus of the study. To account for this, commute patterns for the employees were also studied to determine the region where employees of these parks originated. The commute patterns were obtained from the Census Bureau. Most employees of the industrial park were from the Decatur metropolitan statistical areas (MSA), and for the research park, employees were from the Huntsville MSA. This further confirmed the choice of MSAs for the study. However, the surrounding areas are equally important in providing employees for the respective parks. To account for this, the regions chosen for the economic impact study were the MSAs as defined by the Bureau of Labor Statistics (BLS). The BLS defines the Decatur MSA as the counties of Morgan and Lawrence, and the Huntsville MSA as the counties of Madison and Limestone. To get a more complete picture of the leakages, the economic impact for the two parks for state of Alabama was also undertaken.

IMPLAN, software that is used to study economic impacts, was used in this study. This software also produces the top ten industries affected in terms of employment and output. Though IMPLAN produces the tax effects, a separate method was used to calculate the taxes generated by both parks. After consulting with an accountant, the IMPLAN tax impact results were categorized according to the type of tax and the recipient of the tax. Sales and property taxes were each divided based upon the relative proportion that the county and state respectively share.

III. Results

The results of the economic impacts are presented in two major parts. First, the economic impacts of the two parks at the MSA level are presented, followed by the impacts at the state level. As mentioned earlier, the indirect and induced effects are presented as the multiplier effect. The income column represents the total labor income including compensations. The value added column is the regional equivalent of the national GDP. The output column presents the total output generated including the intermediate purchases.

MSA Level Results

The total number of firms at MFCIP is estimated to be 15 and at CRP, 204. Each firm was placed under a major category according to the output produced by that firm. For instance, the United Launch Alliance, which produces rocket components, was categorized under Propulsion units and parts for space vehicles in MFCIP. There were five (5) categories for MFCIP and twelve (12) categories for CRP. The MFCIP results are presented first at the MSA level. This includes Morgan County and Lawrence County. Table 1 presents the employment, income, value added and output effects for MFCIP.

The direct impact for employment for MFCIP is 1,560 workers, and the total impact for MFCIP for employment is 2,752 workers. The direct impact for output is \$747 million for MFCIP and the total impact for output for MFCIP is \$907 million. The total tax impact for MFCIP at the MSA level is over \$29 million.

Figures 1a and 1b show the breakdown of the firms at MFCIP. The top five industries are presented along with the output and employment in those five industries that are affected by MFCIP. Steel and propulsion units are the main contributors to MFCIP's impact followed by chemicals and plastics and graphite manufacturing. Figure 1c is a graph that shows employment and output for the top six industries. For instance, propulsion units produces over \$240 million in output and employs about 850 workers. On the other hand, rolled steel manufacturing produces over \$167 million in output but employs only about 165 workers.

Figure 1c illustrates that the ratio of output per employee differs between industries, e.g. rolled steel manufacturing produces more output per employee than the propulsion units industry. Since the propulsion units industry involves significant research and development, output per employee is markedly lower than the other industry ratios in the MFCIP.

The industries in the MFCIP are primarily concerned with manufacturing, so in general, the output per employee ratio helps illustrate how manufacturing industries result in employment that produces taxable goods. Table 2 presents the economic impact of CRP. It shows that the direct employment at CRP is 18,744, and total employment effect for CRP is 34,047. The direct impact of output at CRP is about \$3.8 billion, and total impact of output is a little over \$5.7 billion. The total taxes impact for CRP is about \$117 million.

Table 1 Economic Impacts of MFCIP (MSA)						
Impact Type	Direct Effect	Multiplier Effect	Total Effect			
Employment	1,560	1,192	2,752			
Labor Income (millions)	\$150	\$45	\$195			
Value Added (millions)	\$245 \$82 \$327					
Output (millions)	\$747	\$160	\$907			
Sales and Other taxes and fees	d fees \$18,087,23					
Property taxes	\$8,94					
State Income			\$2,186,414			
Total			\$29,214,811			

Figure 1a: Employment by category for MFCIP (MSA level)

Figure 1b: Output by category for MFCIP (MSA level) (millions)







Table 2 Economic Impacts of CRP (MSA)						
Impact Type	Direct Effect	Multiplier Effect	Total Effect			
Employment	18,744	15,304	34,047			
Labor Income (millions)	\$1,536	\$660	\$2,197			
Value Added (millions)	\$1,890	\$1,126	\$3,016			
Output (millions)	\$3,793	\$1,946	\$5,739			
Sales and Other taxes and fees			\$71,255,067			
Property taxes	\$36,089,563					
State Income Taxes			\$9,900,238			
Total			\$117,244,868			





Figure 2c: CRP Top 6 Industries by Employment (MSA)

MSA CRP Output MSA CRP Employment

Figure 3b: Output by category for

MFCIP (State level) (Millions)

Machinery

Repair, \$4

Steel,

\$1,888

Propulsion Units, \$350_

Carbon and Graphite

Manufacturing, \$149

Chemicals and

Plastics, \$156

Table 3 Economic Impacts MFCIP (State) (in millions)					
Impact Type	Direct Effect	Multiplier	Total Effect		
		Effect			
Employment	2,295	4,780	7,075		
Labor Income (Millions)	\$231	\$239	\$470		
Value Added (Millions)	\$477	\$441	\$918		
Output (Millions)	\$1670	\$876	\$2,546		
Sales and Other taxes and fees			\$20,432,690		
Property taxes	\$1,761,138				
State Income	\$7,132,948				
Total	\$29,326,776				

Figure 3a: Employment by category for MFCIP (State level)



Figure 3c: MFCIP Top 6 Industries by Employment (State) 1200 1000 900 1000 Output (Millions) 800 700 Employment 800 600 600 500 400 400 300 200 200 100 0 0 Iron and steel Propulsion units Wholesale trade Truck Carbon and Employment mills and and parts for transportation graphite product services ferroalloy space vehicles manufacturing manufacturing and guided missiles manufacturing

Table 4 Economic Impacts of CRP (State)						
Impact Type	Direct Effect	Multiplier Effect	Total Effect			
Employment	\$18,746	\$19,442	\$38,188			
Labor Income (millions)	\$1,302	\$817	\$2,118			
Value Added (millions)	\$1,655	\$1,386	\$3,041			
Output (millions)	\$3,559	\$2,474	\$6,032			
Sales and Other taxes and						
fees			\$70,781,704			
Property taxes	\$4,554,995					
State Income	\$32,298,505					
Total	\$107,635,204					

Figure 4a: Employment by category for CRP (State level)

Figure 4b: Output by category for CRP (State level) (Millions)







CPR total state Output CPR total state Employment

The top industries in CRP are serviceoriented industries. Figures 2a and Figure 2b show the top five industries in terms of employment and output at CRP. Electronics, engineering services, and research & development are the top three contributors for the CRP. Figure 2c is a graph that depicts output and employment in the top 6 industries. For instance, Architectural and Engineering Services employs about 4,500 workers and produces over \$680 million in output, and Research & Development employs about 3,500 and produces over \$765 million.

For the industrial park, steel firms comprised the majority of output. However, the aggregate output of the steel firms is comparable to that of the United Launch Alliance (ULA), a rocketry joint-venture between Boeing and Lockheed Martin. The ULA, in fact, is responsible for almost half of the MFCIP's employment impact. Unsurprisingly, the research park contains many research and development firms, as well as many electronics and engineering firms. These firms comprise the majority of employment and output effects for the research park. The CRP provides an example of the indirect impact of industries; management services, computer services, trade, and marketing services all observe significant portions of the CRP's employment and output. Interestingly, however, both parks contain steel/metal industry firms, and in both cases, the highest employment multipliers by sector were observed in this category.

State Level Results

Table 3 presents the economic impact of the MFCIP at the state level.

The direct impact for employment for MFCIP is 2,295 workers, and the total impact for MFCIP for employment is 7,075. The direct impact for output is \$1.67 billion, and the total impact for output is \$2.55 billion. Figures 3a and Figure 3b show the top five industries in terms of employment and output at the MFCIP. Steel industries have a significant impact at the state level, with iron and steel mills alone having direct impact of over \$1.1 billion and employing over 900 workers. Furthermore, steel industries in MFCIP observe a significantly higher output per employee than any other industry at the state level.

Table 4 presents the economic impact of CRP. It shows that the direct employment at CRP is 18,746, and total employment effect for CRP is 38,188. The direct impact of output at CRP is about \$3.6 billion, and total impact of output is a little over \$6 billion. Figures 4a and Figure 4b show the top five industries in terms of employment and output at CRP. Not much changes from the MSA level to state level with respect to shares of impacts, as electronics, engineering services, and research & development once again share a majority of employment and output. Figure 4c is a graph that depicts employment and output in the top 6 industries. Notably, output for employees is higher in research & development, wholesale trade, and navigation instruments manufacturing, but lower in engineering services, management services, and employment services. For instance, the navigation instruments industry generates almost \$600 million in output with just over 1,600 employees, while employment services generates about \$86 million with over 2,200 employees.

While the top industries do not change significantly when the state level is considered, their relative portions of total employment and output do. In the MFCIP, for instance, the total effect of the steel firms on the entire state commands the majority of both employment and output. The CRP, meanwhile, observes electronics making larger gains in output from the MSA to state level relative to the other industries and similar relative levels of output in each industry.

Compared to the preceding MSA results, one may observe that the employment multipliers' relative magnitudes change significantly. Indeed, the industrial park observes an employment multiplier greater than two (2) at the state level, while CRP is just greater than one (1). Moreover, the direct effect of the MFCIP on employment changes by several hundred workers from the MSA level to the state level, which suggests that a significant portion of the workforce of those employed at the industrial park do not necessarily live in its MSA, while a similar effect is observed in the research park for indirect effects, suggesting that CRP employees generate significant demand for goods and services available outside of the Huntsville MSA.

Tax Analysis Results

Table 5 Tax Impact per Firm						
	MFCIP	CRP				
Sales and Other	\$1 459 478	\$346.969				
Property taxes	\$125,796	\$22,328				
State Income	\$509,496	\$158,326				
Total	\$2,094,770	\$527,624				

IMPLAN provides tax estimates for taxation. These estimates have been reorganized to aid clarity.

Sales and property taxes were each separated based upon the relative proportion that the county and state respectively share. Finally, the taxes were adjusted based upon the number of firms in the parks. Rounded totals from these modifications are included below in Table 5. Per firm, the MFCIP generated almost \$150 thousand in overall taxes, while the CRP generates about \$2,600. In each category, and overall, the taxes generated per firm in the industrial park exceed those generated per firm in the research park.

This is consistent with the idea that manufacturing and retail industries generate more tangible goods, while service industries do not produce goods that are taxed. However, as seen above, some industries generate more employment, and some industries generate more output. Some industries generate more taxes than others. The recommendation for local governments from this study is to diversify the industries in parks and to offer a variety of employment and output to maximize benefits for its citizens.

IV. Conclusion

The top industries that are most affected by the industrial park are manufacturing based, while the top industries affected by the research park are all service based industries. Furthermore, while the three industries in the industrial park make up most of the employment, research park employment is widespread across a number of industries.

City and county governments need to understand the effect of different industries and the kinds of development that occur with these industries. This study seeks to give an overview of how the presence of these two parks has affected their respective communities and the state of Alabama overall. The different types of parks bolster different industries so for a balanced development of the region, both industrial and research parks are needed. Both provide various types of employment opportunities for their surrounding populations. Additionally, local governments can develop educational and vocational institutions needed to sustain their region's industrial and research parks. Indeed, Erin Koshut, the director of the CRP, has indicated that the research park intends to diversify and develop by attracting new industries and firms

Furthermore, some intriguing comparisons between the MFCIP and CRP can be made. First, the MFCIP appears to be a significantly greater generator of consequential employment relative to its size in its region and the state, while the CRP appears to be a greater generator of output. This distinction, however, manifested itself in statewide results more so than countywide ones. Meanwhile, both parks host steel manufacturing industries that induce large employment multipliers despite the otherwise distinct distributions of industry types. Therefore, there may be more to learn about the differences in economic impact between industrial parks and research parks located in neighboring regions of a common state.

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Source Credibility and Cybersecurity Behaviors

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Abstract – In an increasingly interdependent society characterized by omnipresent online communications, Information Systems security research is an important contributor in helping protect people and organizations from cyber-attacks. Cyber-attacks are increasing in their number and scope. In May 2017 a series of ransomware attacks affected hundreds of thousands of computers across the globe, causing significant loss of business. Understanding how people interact with IT threats is an integral step to cyber-security. In this paper, I modify and extend the model developed by Liang and Xue in their Technology Threat Avoidance Theory to evaluate the effects of source credibility on computer user's behaviors.

I. Introduction

Cybersecurity is an umbrella term that relates both the technological and human domain. Because of this it is crucial to examine the extent of the impact the two have on each-other. To understand this connection it is important to examine the role of human behavior in cybersecurity attacks. Most victims' computers were not updated, or were running outdated software. Organizations may have standing policies in place dictating IT behaviors or systems, but so long as people do not act on these protocols, security vulnerabilities will persist.

It is this vulnerable element that IT security and information assurance literature seeks to understand. While the technical infrastructures underlying security issues are generally well understood, less well developed is research on the role human behavior and communications play in Cybersecurity (Zafar and Clark, 2009). The most significant development in recent decades is the ubiquity of the internet and its impact on communications and society in general. Internet communication is increasingly characterized by the pervasive use of social media throughout the world.

Social media like Facebook, Twitter, YouTube, Instagram, and other platforms, influence the way people communicate and interact with each other, exchange ideas, and support causes and campaigns (Siemans 2005). Communication in social media is of particular interest in marketing research, which has examined how consumers and brands interact with each-other. Social media communication is interesting in that studies have found that online communications (electronic word of mouth) differ from standard word of mouth communications. This distinction has important implications in IS research, and was a critical component as we constructed our research model.

II. Background and Related Work

In developing a Technology and Source Credibility model we considered the role social media might play in impacting Cybersecurity knowledge transfer, and how the characteristics of social media and social media networks enable this (Gupta, J; Patnayakuni, N; Patnayakuni, R., 2017). To approach this and build constructs for our model, we draw from two behavioral models from prior research: the Technology Threat Avoidance Theory – TTAT, and the Elaboration Likelihood Model – ELM. (Liang and Xue 2009; Cacioppo, Petty 1986).



Figure 1: Conceptual Model: (Gupta, J; Patnayakuni, N; Patnayakuni, R., 2017)

The TTAT model explains how individual users avoid IT threats in voluntary settings (Liang and Xue, 2009). Liang and Xue (2009) argue that security behaviors users engage in are a two part process that distinguishes threat appraisal from coping appraisal. Further, the TTAT posits that users are influenced by different mechanisms in each process. Threat appraisal is distinguished from coping appraisal based on the discrepancy between the two states. That is, the difference between the two is the dissonance created by an acknowledged threat and the impact it could have on the user. These distinctions are defined as driving individual attitudes and behaviors towards IT threats.

In identifying this difference, Liang and Xue (2009) developed a model using eight constructs: perceived severity, perceived susceptibility, perceived threat, safeguard effectiveness, safeguard cost, self-efficacy, avoidance motivation, and avoidance behavior. Liang and Xue (2009) hypothesized the relationships between these constructs in their model, and the empirical support they found for their model is the principle reason why we adopt the TTAT model for this summer's research.

We extend the TTAT model of the threat appraisal process by adopting concepts from the Elaboration Likelihood Model into our Source Credibility model. The ELM is of interest to us in how it examines the influence of source credibility in the context of different communication types. The ELM examines cognitive processing based on how individuals respond to persuasive messages (Cacioppo, Petty 1986). Considering the important role communication plays in influencing behavior we propose that people evaluate cybersecurity information differently in the online context than traditional news media when making appraisals of individual vulnerability. We propose that this can be evaluated using constructs adopted from the TTAT and ELM, and examined in the context of source credibility.

We define source credibility as the functional image of the source in the minds of the receivers. Studies demonstrate a strong correlation between source credibility and behavioral influence in standard communication transactions (Dholakia & Sternthal, 1977; Bansal & Voyer, 2000). As behaviors are the primary drivers behind responses to perceived threats, we adopt the TTAT's model for our evaluative purposes.

III. Method

To test the hypothesis a questionnaire was developed that evaluated the characteristic behaviors of individuals when confronted with cybersecurity information. To distribute our questionnaire we used the online survey tool Qualtrics. Our survey was disseminated through email to all the students in the College of Business. We sent out 1223 emails, of which 489 were opened. From the opened emails we had 164 respondents. For the purposes of a pilot study, this 34% response rate was acceptable. The overall response rate of all respondents contacted, 13.4%, is also very respectable. In the survey, two cybersecurity scenarios were created. One scenario identified the source of cybersecurity threat information as standard news media information. The other identified the source of cybersecurity threat information as social media for the same IT threat. To measure users' responses to our constructs, we used a modified Likert scale, as well as a personal inventory metric.

The first scenario we presented users with is as follows:

"You receive the following news update from print media (for example The Wall Street Journal) or News and Cable news networks (for example NBC):

Malicious software known as ransomware has been making headlines after hackers hijacked hundreds of thousands of computer worldwide. Ransomware locks up user's data and threatens to permanently delete the data if a ransom is not paid. The global impact has been across more than 150 countries across America, Europe and Asia.

You have also heard that at least 66 computers on the UAH campus have been affected by the attack."

The second scenario we presented users with is as follows:

"You receive a post from a member of your social media circle on one of your social media channels (for example Facebook):

Massive ransomware attack! One of my friend's computers has been infected! She can't access her data! Pass on the information to everybody you know and ask them to be careful out there. Go to this site http://clover.vessel.com/wash/r3c5/mal.aspx to learn how to protect your computer.

You have also heard that at least 66 computers on UAH campus have been affected by the attack."

This questionnaire had constructs adopted from Liang and Xue's (2009) Technology Threat Avoidance Theory and the Elaboration Likelihood Model. These constructs were: perceived susceptibility, perceived severity, perceived threat, avoidance motivation, avoidance behavior, perceived safeguard cost, and perceived safeguard effectiveness. In addition to this, we designed constructs measuring self-efficacy, issue involvement, and source credibility.

Our first three constructs were perceived susceptibility, perceived severity, and perceived threat. In our study we defined perceived susceptibility as an individual's subjective probability that a malicious IT would affect him or her. Put in the context of each scenario, we attempted to identify the strength of response to an itemized list of questions concerning this definition and how likely the individual felt they would be exposed to an IT threat.

Related to the construct of perceived susceptibility is the construct of perceived severity, which is defined as how an individual perceives the negative consequences of an IT threat. We measured this by a series of questions which examined how users felt about loss of personal information, property, or financial assets. Finally, we defined our perceived threat construct as the extent to which an individual perceived a malicious IT as dangerous or harmful. We asked people how they felt the likelihood they would be affected by the IT threat was based on the scenario they were given.

In addition to these we had three constructs measuring users' perception of safeguard measures and personal competence. In the context of these, they were: perceived safeguard effectiveness, self-efficacy, and perceived safeguard cost. We defined perceived safeguard effectiveness as an individual's subjective assessment of how effectively a given safeguard measure – such as spam filters, anti-virus, windows updating, is against a given IT threat.

With safeguard effectiveness we also designed a construct that attempted to measure a user's self-efficacy, or confidence in enacting these safeguard measures against IT threats. Finally, we designed a construct to measure safeguard cost. We defined safeguard cost as the physical and cognitive efforts required by an individual to use safeguard measures. Our intention was to evaluate individual perceptions of investment in dealing with IT threats. The final constructs we adopted from the TTAT for our model were avoidance motivation and avoidance behavior. Avoidance motivation is defined as the degree to which IT users are motivated to avoid IT threats by taking safeguard measures. We asked survey participants if the scenario they were given influenced their security behaviors. These security behaviors are captured in the avoidance behavior construct, which is defined as the extent to which individual motivation influences action against IT threats.

Last, we created a construct measuring users' issue involvement. We adopted this construct from McQuarrie and Munson's (1987) personal involvement inventory, and defined it as the extent with which an individual user is engaged with cybersecurity. In designing this, we hoped that a strong involvement would correlate to a higher net avoidance motivation and behavior.

In addition to collecting data on these constructs, we collected general demographic information on survey respondents. This data included: gender, class standing, major, number of credit hours taken each semester, computer and internet usage, and educational experience of parents.

IV. Results and Analysis

A series of statistical analyses were performed on our data. Using Excel, R, and SPSS we examined our data using a variety of statistical techniques, and for the purpose of this paper relied primarily on correlation analysis. We summarized our most important findings by their r values. Our findings are represented by the following correlation matrix. In the matrix the r value for each construct is given in relation to another construct. R values denote the strength and direction of correlation between two variables in a linear relationship. While accepted or expected r levels vary by discipline and topic, studies like ours report a strong relationship for values of .7 or higher, and a moderate relationship of .5 or higher. The high r values displayed in our correlation matrix signify a need for more investigation on our model and research in this area.

In our data we found that source credibility was not found to have any noticeable effect on our model's constructs (no statistically significant r value above .157), but other worthwhile findings emerged (Figure 1). Not only did each scenario test differently, but pronounced relationships were found between several of our constructs.

First, we found a strong correlation of .808 between perceived susceptibility and perceived threat. We also found a suggestive r value of .564 between our self-efficacy and avoidance behavior constructs and an r value of .526 between our self-efficacy and avoidance motivation constructs. Furthermore, we found a strong positive correlation between avoidance motivation and avoidance behavior of .77. Finally, indicative r values of .553 and .459 were found between our issue involvement, avoidance behavior and avoidance motivation constructs. Taken together, these r values suggest a moderate to strong positive correlation between the mentioned constructs (Figure 1).

Other interesting relationships also emerged from the correlation matrix. The perceived severity and perceived susceptibility constructs had an r value of .384. As well, perceived severity and perceived threat had an r value of .375 (Figure 1). Though these have no effect on our hypothesis, these lower values may provide interesting avenues for future exploration and research.

	1	2	3	4	5	6	7	8	9	10
1. Source Credibility	1	-0.004	-0.172	-0.027	-0.109	-0.158	0.157	-0.181	-0.206	-0.067
2. Perceived Susceptibility	-0.004	1	0.808	0.384	0.038	-0.284	0.235	-0.013	-0.002	0.196
3. Perceived Threat	-0.172	0.808	1	0.375	0.038	-0.284	0.23	0.118	0.09	0.318
4. Perceived Severity	-0.027	0.384	0.375	1	0.254	-0.083	0.192	0.084	0.109	0.21
5. Safeguard Effectiveness	-0.109	0.069	0.038	0.254	1	0.27	0.05	0.3	0.379	0.066
6. Self Efficacy	-0.158	-0.333	-0.284	-0.083	0.27	1	-0.5	0.526	0.564	0.198
7. Perceived Avoidance Cost	0.157	0.235	0.23	0.192	0.05	-0.5	1	-0.433	-0.408	-0.203
8. Avoidance Motivation	-0.181	-0.013	0.118	0.084	0.3	0.526	-0.433	1	0.77	0.553
9. Avoidance Behavior	-0.206	-0.002	0.09	0.109	0.379	0.564	-0.408	0.77	1	0.459
10. Issue Involvement	-0.067	0.196	0.318	0.21	0.066	0.198	-0.203	0.553	0.459	1

Figure 2: Correlation Matrix

V. Conclusion

Future models for research like this should more strongly distinguish the constructs between individual scenarios. Due to significant time constraints it was impossible to design, test, refine, and analyze data from our model in a desired fashion. Different and more thorough statistical analyses might provide more revealing information about our data sets. Second, our survey was constrained to a small population of business students at the University of Alabama in Huntsville. As such, it is possible our results are not representative of a wider demographic. Differences we noted in scenario treatments might be more pronounced, or more suggestive provided a larger and more diverse sample set. Finally, it is possible our treatment of our individual constructs and women across our issue involvement constructs is listed. Each data point shows the disparity of responses between men and women.

This data may provide useful avenues of research in the future. Currently, there is a paucity of IT literature on gender involvement in cybersecurity. If our sample data is representative of the larger population, understanding why this disparity exists, and what mechanisms underlie it could prove useful in constructing cybersecurity responses to threats that considers both men and women's engagement.

Cybersecurity is a cybernetic process. It should consider both the hardware capital and human resources available. In the future, creating a secure environment in which governments, businesses, and



Figure 3: Gender Differences in Cybersecurity Responses

could be adjusted to make user evaluation of source credibility in response to each scenario more meaningful or personal.

In spite of the data not immediately supporting our source credibility model, some meaningful observations emerge from the larger dataset in the context of demographic information collected. For example, an analysis of the constructs between genders shows that each scenario was treated differently. Further, a significant difference exists across the strength of IT engagement between genders. Women were found to be less involved and confident in enacting safeguard measures for IT threats. In the following chart the mean score of responses for men individuals interact will require research in both the computer and behavioral sciences. No matter how efficient or failsafe a technical infrastructure is, human behavior will remain an integral part of IT security. Current consumer research – such as the use of big data in targeting customers, may be useful to businesses and organizations in understanding the IT behaviors of its employees or constituents. A successful IT infrastructure in the future may constitute of individual training armed by data in addition to anti-virus software, spam filters, and firewalls.

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Validation & Optimization of CAPTURETM:

Rapid Diagnosis of Pathogens Causing Urinary Tract Infections

Elizabeth Marie Richards Gates College of Nursing

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Abstract

Background – The number of bacterial strains resistant to antibiotics is growing exponentially. Antibiotics are often prescribed more than needed due to the delay period in diagnosing the pathogen and giving treatment. This delay often pushes prescribers to give a broad-spectrum antibiotic or pushes them to make the patient wait for the proper treatment, in turn allowing the bacterium to potentially mutate. This creates a need for a more rapid, easily used, and cost effective method of identifying pathogens. The objective of this study is to validate the CAPTURETM method and its ability to identify pathogens, reduce the mechanical processing time, and optimize the sample preparation and lysis protocols.

Methods – The methods for this project included several steps: captor design, sample acquisition, sample prep, sample testing using the CAPTURETM method, assay analysis, sample comparison, and optimization. Urine samples were gathered from patients with suspected urinary tract infections at the University of Alabama in Huntsville's Faculty & Staff Clinic. Each patient consented to give two urine samples, one sample was sent to the facility's contracted lab for routine identification. The second sample was sent to GeneCapture and considered discarded and de-identified. Institutional Review Board approval was received and is on file from the University of Alabama in Huntsville.

Results – To determine the accuracy of the CAPTURETM assay pathogen identification, the results from the contracted lab were compared to the results of the CAPTURETM assay. The results showed that the CAPTURETM method has the ability to identify pathogens from the lysates.

I. Introduction

The purpose of this research study is to test the validity and reliability of the CAPTURETM method. The CAPTURETM was created by GeneCapture, Inc., a start-up company in the Biotechnology field. The CAPTURETM (Confirming Active Pathogens Through Unamplified RNA Expression) method uses a molecular diagnostic technique to determine a genetic match for the pathogen using DNA captors that will identify the pathogen in less than one hour for minimal cost. CAPTURETM's purpose is to rapidly diagnose bacterial and viral pathogens. The need for a more rapid diagnostic testing method continues to grow exponentially as antibiotic resistance increases, strains mutate, and cost of healthcare rises.

To begin the study, a partnership was cultivated between GeneCapture, Inc. and the University of Alabama in Huntsville's Faculty & Staff Clinic, a division of the College of Nursing. Institutional Review Board approval was received from The University of Alabama in Huntsville (Appendix A). Patients that presented symptoms of a urinary tract infection (the phrase urinary tract infection will be further noted as UTI in this text) were recruited to participate in the study and consented to give two urine samples (Appendix B, 1) (Appendix B, 2), one to be run through the CAPTURE[™] method and the other sent for a culture and sensitivity to the contracted laboratory for usual diagnostic tests and treatment. The anticipated outcome of this research is the verification of CAPTURETM, through comparative results, to correctly identify these common urinary tract pathogens. These findings may advance further into other infectious pathogens and may be useful in correctly and rapidly diagnosing common to serious infections in clinics and areas with no access to laboratory facilities.

II. Background

PCR

Polymerase chain reaction (denoted as PCR through the rest of the text) is a technique used in many laboratories and development companies worldwide. This technique was invented in the 1980s and has been making many advances in recent decades. PCR allows a particular DNA region, selected by the researcher, to be targeted and copied ("Polymerase Chain Reaction", 2017). Generally, a researcher would want multiple copies of a specific region to analyze it for certain characteristics or functions or pathogen identification (Järvinen, 2009).

For PCR to function, a DNA polymerase enzyme is required to make new strands of DNA. Taq polymerase is the most common DNA polymerase used for PCR. This particular polymerase is derived from a heat-tolerant bacterium, making it ideal in PCR. PCR uses high temperatures at many points in the process to denature the template DNA ("Polymerase Chain Reaction", 2017). For the polymerase to work, a primer is needed. Two primers are used in a PCR experiment; these primers are short pieces of single-stranded DNA. Each primer is tasked with surrounding the target region of DNA and binding to opposite strands of the template DNA, leaving the target region unbound ("Polymerase Chain Reaction", 2014). When the strands are cooled, the primers are allowed to bind to the template. Once they are bound, the temperature is increased allowing synthesis of new DNA.

This process occurs 25-35 times in a single PCR experiment. Each experiment takes between two and five hours. The time will depend on the length of the DNA sequence that is targeted and being copied. ("Polymerase Chain Reaction", 2014) After the reaction is complete, gel electrophoresis is often used to visualize the reaction. This process takes between 1 and (Järvinen, 2009) 1.5 hours ("Agarose Gel Electrophoresis", n.d.). PCR can also be analyzed on a microarray (Järvinen, 2009). Often, when analyzing a PCR reaction, a researcher may find unexpected amplifications. A limitation of PCR is its need for sample purity. If a contaminant is involved in the reaction, the contaminant may be copied as well, skewing the results (Brookman-Amissah, 2014). Extra steps to prevent contamination between samples must be taken.

САРТИRE^{тм}

Confirming Active Pathogens Through Unamplified RNA Expression (the phrase will be further noted as CAPTURETM in this text) is a method developed by GeneCapture, Inc. to be used as a rapid diagnostic tool. CAPTURETM uses a stem-loop captor to identify pathogens. The stem is a specific sequence that does not change. The loop is a unique sequence from the pathogen(s) that the specific panel is trying to identify.

The CapLab is the machine, designed by GeneCapture, to carry out the CAPTURETM process. A lysate from a sample (in this case from urine) was placed in a cartridge along with buffers and targets. The sample is run across an array of appropriate stem-loop captors. If the sequence of the sample finds a match in one of the loops, it will bind. Once the sample binds, the loop is forced to open and an oligonucleotide binds to the hanging stem. This oligo acts as the detector, producing the signal needed to analyze (Boeteng, 2013). (See Figure 1)



Figure 1: Pathogenic nucleic acids pass over the captors on the microarray and bind to their complement in the loop region; this binding forces the stem to open (the captors only remain open if the correct target has bound). The captors that remain open then bind to a universal detector. The microarray is washed stringently to remove any mismatched or unbound nucleotides (Koelle, 2014.)

After this process, the array is cooled back down to allow the loops with no binding to close. The microarray can then be analyzed for the specific pathogen (Boeteng, 2013). (See Figure 2)





Figure 2: Microarray. Identifies which captors bound to the sample. The fluorescent detector shows the signal from each bound captor. The 'landing lights' allow the person analyzing to orient the direction of the array and to correctly identify which captors bound. (Koelle, 2014.)

CAPTURE[™] looks at expressed RNA rather than rDNA. It has the ability to target hundreds of pathogens in a single assay (Pusey, Chittur, 2007). Captors do have the potential to cross-react with other species but statistical cluster analyses are completed to counter this possibility. For more information regarding the specific binding processes and hybridization, refer to Novel stem-loop probe DNA arrays: Detection of a specific acetotrophic 16s ribosomal RNA signatures, 2012, Jonas Boateng, Robert Zahorchak, Joel Peek, & Krishnan Chittur.

	PCR	CAPTURE TM
Disease Coverage	Looks at one pathogen at a	Targets hundreds of pathogens
	time	in a single assay
Specificity	Sample purity is key, contaminates are often	Crude lysates from any matrix (urine, blood, saliva)
	amplified or duplicated	
Cost	>\$100/test	~\$20/test
Sample Turn-Around-	few hours to days	Currently 1 hour, goal <30
Time		minutes
Primer Binding	rDNA	Expressed RNA

PCR & CAPTURETM Comparison

In this table, the CAPTURE™ diagnostic is compared to multiplex PCR

UTIs

Many factors, lifestyles, and diagnoses such as an active sexual life, menopause, diabetes, difficulty emptying the bladder, or obstruction may lead to UTIs. All of these factors influence the high occurrence rate of UTIs, especially among women. Research has shown that 40 to 60 percent of women will have at least one urinary tract infection in their lives (Stapleton, 2017). Male and female UTIs add up to approximately 150 million cases worldwide per year related with an estimated \$6 billion dollar healthcare expenditure (Baldato, 2016). Due to the rate of occurrence, ease of access, and the need for rapid identification, this study chose to collect and analyze suspected UTI samples.

The current method for identifying pathogens in human urine samples is a lengthy process, usually taking two to three days to complete. First, a clean catch midstream void is collected and then a dipstick urinalysis is performed, ending with a quantitative urine culture completed by a lab (Baldato, 2016). Due to this delay in a specific diagnosis of the infecting pathogen, a broad-spectrum antibiotic is often given (Zeeman, 2007). If the culture results differ from the original speculation and the antibiotic given does not hinder the pathogen identified as the infectant, the patient has received an unnecessary treatment that could later lead to antibiotic resistance (Ventola, 2017). Additionally, if the pathogen is not identified rapidly, the pathogen has the potential to mutate and move, causing acute pyelonephritis (kidney infection) ("Urinary Tract Infections", 2017).

Antibiotic Resistance

Antibiotic resistance is the innate or acquired ability of bacteria to resist the effects of antimicrobial agents. Acquired resistance is of more concern due to the possibility of rendering currently effective drugs, ineffective (Burchum, 2016). Over time, bacteria may become less sensitive to an antibiotic or may lose all sensitivity (Burchum, 2016). Antibiotic resistance is increasing due to overuse and misuse of antibiotics ("Get Smart", 2014), extended hospitalization (Burchum, 2016), and antibiotic use among food sources i.e. giving animals antibiotics ("Get Smart", 2014). Research studies have shown that 30 to 50 percent of antibiotics given were incorrect for the infection or the duration of the treatment (Ventola, 2017).

Antibiotics are only effective against bacteria, not viruses or fungi. These agents are not effective against common colds, gastrointestinal viruses, the flu, most sinus infections, or ear infections ("Get Smart", 2014); however, they are often prescribed for these diagnoses due to delayed diagnostic testing to identify the specific infecting pathogen, patient knowledge deficit, and improper provider prescriptions.

III. Methods

Captor Design

Captor design is crucial to the functionality of the CAPTURETM method. The purpose of the captor design process is to identify a sequence unique to each pathogen with little to no crossover with other species. Some pathogens fall in the same phylum or genus but still need to be differentiated to come to a proper diagnosis. There is still the potential of crossover between species; therefore, statistical cluster analyses were completed to account for the potential interactions. GeneCapture owns a proprietary software program that utilizes uploaded genomic information of the most highly expressed genes to seek out sequences that are common to an individual pathogen but also retain unique areas that set it apart from other any others. The sequences are then attached to the 'universal stem' and placed on the array. Though the captor design process looks for a unique sequence for each pathogen, it still must take into account the constantly changing nucleic acids in each organism. For this reason, multiple captors are designed for each pathogen and placed on the array; up to 5 captors will be used for one pathogen. Positive and negative controls are included on each array.

Sample Acquisition

Samples were gathered from patients with suspected UTIs that presented to the University of Alabama in Huntsville's Faculty & Staff Clinic. The staff of the clinic asked the patients if they would give two samples, one for regular lab testing and one to be given to GeneCapture for testing. Patients were made aware that none of their personal information would be given to GeneCapture or be used for this study. The samples were considered discarded and de-identified. Samples were kept in a refrigerator at the clinic until time of acquisition. The samples were then transported, by a GeneCapture staff member, to the lab in a marked biohazard container.

Sample Prep

To prepare each sample received from the clinic, a specific series of manual steps were followed. In this process a lysis buffer was used to break open the cells of the pathogen and fragment the RNA. (The specific process and lysis buffer have been excluded from this text for proprietary reasons). After the steps were completed, a sample of the lysate was plated to confirm sterility. In addition, through dilution plating, a portion of each original urine sample was used to confirm and compare growth and identity of the pathogen with the results received from the clinic's contracted laboratory.

Sample Testing

The lysate along with the buffers (the specific buffers have been excluded from this text for proprietary reasons) are paced in a cartridge designed specifically for the CapLab. The machine uses a simple pump, like that used in a common aquarium, to move a succession of fluids (the lysate and buffers) across the array. As the fluid moves across the array, the target (lysate) binds to the corresponding captors. A rinse is then completed to wash away any excess target. The fluorescent detector will then bind to the open captors and another rinse will be completed. A final rinse is completed to stabilize duplex formation before scanning.

Assay Analysis

The future of the CapLab will allow the assay analysis and, therefore the identification of the pathogen, to be completed within the CapLab itself. (See Figure 3).



Figure 3: Vision for future CapLab design.

During this study a GenePix 4200b Scanner (Molecular Devices, Inc, Sunnyvale, CA) was used to scan the resulting microarrays containing the now closed captors and bound florescence. (See Figure 2). With the map of the placement of each captor, the pathogen was identified from the signal on the array.

Comparison

Once the assay was analyzed and the infecting pathogen determined by the CAPTURETM method, the results were compared to the results given by the University's contracted lab. The results from the lab took 2-3 days to receive.

Optimization

After each sample was tested and analyzed, the process was scrutinized for areas that needed improvement due to overlap, lack of specificity, and chemical change. During the time that this particular study was completed, the lysis protocol was updated several times and captors were redesigned.

IV. Results

Sample	+/-	ID	+/-	ID
UAH001	(+)	Group B Strep	(-)	
UAH002	(+)	E. coli	(-)	
UAH003	(-)	Mixed flora	(-)	
UAH004	(-)	No growth	(-)	
UAH005	(+)	E. coli	(+)	E. coli
UAH006	(-)	No growth	(-)	
UAH007	(-)	No growth	(-)	
UAH008	(-)	No growth	(-)	
UAH009	(-)	No growth	(-)	
UAH010	(-)	Mixed flora	(-)	
UAH011	(+)	Group B Strep	(-)	
UAH012	(-)	Mixed flora	(-)	
UAH013	(+)	E. coli	(+)	E. coli
UAH014	(+)	K. pneumoniae	(+)	K. pneumoniae
UAH015	(-)	No growth	(-)	
UAH016	(-)	Mixed flora	(-)	
UAH017	(-)	No growth	(-)	
UAH018	(-)	No growth	(-)	
UAH019	(+)	E. coli	(+)	E. coli

Shown above, the results from the contracted lab are compared to the results of the CAPTURETM assay. When a negative result was received from the CapLab and not the laboratory, the captors as well as the lysis protocol were analyzed for areas that needed optimization. Some captors had to be redesigned to have a higher specificity and less overlap with other species. The lysis protocol had to be adjusted to account for samples that yielded very small amounts of lysate.

The CAPTURE[™] results for sample UAH002 did not yield E.coli as the culture results did. It was determined that the RNA extraction process for this sample resulted in too little detectable RNA. E. coli was detected in future samples. Culture results for the samples UAH001 and UAH011 found Streptococcus agalactiae or Group B Strep. Further research showed that this particular bacterium can be a cause of UTIs, a result of a kidney stone and can be very harmful during pregnancy. No captors for this bacterium were on the panel used in this study; after discussion, captors for this bacterium were designed and added to a new panel for future studies.

A lab culture of "mixed flora" indicates that only low levels of multiple bacteria were grown but were not an indicator of infection. The mixed flora result was most likely yielded due to a non-sterile urine sample. The urinary tract and urine are sterile unless contaminated by pathogens. Normal flora are bacteria that live on or in a human body at all times. These bacteria are not pathogenic and aid the body in fighting off harmful bacteria. Every person carries normal flora in areas such as the gastrointestinal tract, nose, mouth, skin, and specifically, around the genitals. Using a clean catch method to obtain a urine sample would eliminate this flora from contaminating a sample; however, patients often complete the clean catch method incorrectly, resulting in a contaminated sample. (See figure 4 for steps to gather a proper clean catch sample)

V. Conclusion

This study, which specifically looked at UTI samples to validate and optimize the CAPTURE[™] method is an ongoing study and is now being conducted alongside other more in- depth studies at the GeneCapture lab. Through this research study, it was determined that the CAPTURE[™] method does have the ability to identify specific pathogens from human samples. It was determined that the CAPTURE[™] method can be completed in one hour with the direction of being completed in less time as more steps in the



Figure 4: *Steps to properly gather a clean catch urine sample.*

process become a part of the automation of the CapLab. Further optimization of the UTI captor panel is currently underway.

The CAPTURE™ method and this study have many implications for medical and nursing practice. This form of rapid diagnostics allows for determination of the infecting pathogen in a clinic and at the bedside for timely and accurate treatment, thereby reducing the exponential growth in the number antibiotic resistant pathogens. This technology has the potential and accessibility to be used in areas with little to no access to laboratories or medical facilities such as: military camps. disaster areas. and wilderness/remote installments. There are possibilities for use in pandemic emergence and tracking. CAPTURE™ can also be utilized in agriculture to identify plant pathogens reducing crop destruction and increasing food insecurity. There is also potential for cancer detection and treatment monitoring. This research is ongoing and it will be exciting to determine how this technology will improve health outcomes, in the future.

Publication of the expanded validation study is expected in the near future.

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Using Bacteria to Eliminate the Glycerin Glut

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Abstract – Crude glycerol is produced in large quantities as a byproduct of biodiesel production. *Clostridium pastuerianum* is an anaerobic species of bacteria capable of using glycerol as its sole carbon source. *C. pasteurianum* is known to convert glycerol into n-butanol, which is useful as a fuel additive. In this study, we measured the concentrations of glycerol along with compounds produced by *C. pasteurianum*, including n-butanol, under varying growth conditions. We found that doubling the initial concentration of glycerol had no noticeable effect on the ability of the bacteria to grow or produce butanol. Based on this finding, we intend to move forwards with a higher concentration of glycerol in order to sustain a continuous culture of bacteria in a bioreactor.

I. Introduction

Biodiesel-derived crude glycerol is produced as a byproduct of biodiesel production at 10% (w/w) [1]. An increase in the amount of biofuel production has led to an overabundance of crude glycerol. The production of more glycerol than there is a demand for, often referred to as the "glycerol glut", has led to an increased demand for an economically feasible method of disposing or using the glycerol [2].

The ability of C. pasteurianum to convert glycerol into butanol, a substance which can be used as a fuel additive, could be the key to eliminating unnecessary waste from current biofuel production methods [3, 4]. This species of Clostridium is of particular interest as it can utilize glycerol as its sole carbon source [1]. In addition, this bacteria can use crude glycerol directly, since the only compounds in crude glycerol that are toxic to this species are fatty acids, which can be easily removed from crude extracts [5]. Clostridium pasteurianum is an anaerobic, non-infectious soil bacterium. It is capable of producing a range of products from glycerol, including acetic acid, 1,3-propanediol (1,3- PDO), ethanol, butyric acid, and butanol, as part of its regular metabolism [4]. Butanol in particular is of special interest due to its miscibility in petroleum, lower vapor pressure, and higher energy content compared to current fuel additives, specifically ethanol [1]. The potential butanol has for improving the efficiency of biodiesel and other forms of biofuel makes it a desirable product. The purpose of this project is to determine trends in the concentration of butanol and other substances generated by *C. pasteurianum* to aid in the eventual design and implementation of a bioreactor capable of sustaining a continuous culture of this species that can consistently convert glycerol to butanol.

II. Materials and Methods

Growth of Bacteria

C. pasteurianum ATCC 6013 strain was obtained from American Type Culture Collection. Frozen samples were thawed and then cultured in glucose media. Glucose media is composed of 3.74 g/L K2HPO4, 1.43 g/L KH2PO4, 2.2 g/L (NH4)2SO4, 80 g/L dextrose, and per 100mL solution: 1mL MgSO₄/FeSO₄ solution (22g/L MgSO₄, 0.55 g/L FeSO₄) per 100mL total solution, and 200 microliters trace metal solution SL7 per 100mL total solution. Trace metal solution SL7 is composed of 10mL of 25% HCl solution per liter, 1.5g/L FeCl₂·4H₂O, 190.0 mg/L CoCl₂·6H₂O, 100.0 mg/L MnCl₂·4H₂O, 70.0 mg/L ZnCl₂, 62.0 mg/L H₃BO₃, 36.0 mg/L Na2MoO4·2H2O, 24.0 mg/L NiCl2·6H2O, 17.0 mg/L CuCl₂· $2H_2O$. 5mL of CaCO₃ were added to 100mL of the glucose media inside of the glove box.

Cultures were grown in a glove box under anaerobic conditions until an Optical Density (OD) of 0.6 and then transferred to glycerol media. Glycerol media has the same composition as glucose media, but 25g/L of glycerol are used instead of 80g/L of dextrose. Flasks were shaken continuously for both growth and fermentation at $37^{\circ}C$.

Fermentations

All fermentations were done in a flask designed to simulate a reactor in a glove box under anaerobic conditions at 37°C. The atmosphere in the

glove box consisted of approximately 5% hydrogen and 95% nitrogen. A pair of palladium catalysts was used to remove excess oxygen from the air by catalyzing the reaction:

$2H_2 + O_2 \rightarrow 2H_2O$

A second pair of catalysts was rotated with the first pair weekly, and the catalysts regenerated. Fans were used to mix the air to optimize the removal of oxygen by the palladium catalyst. The pH was measured each day to determine growth using a pH meter. The optical density (OD) was also measured at 610nm each day.

Taking Samples

Samples were taken in 24-hour intervals from the reactor. These samples were centrifuged at 10,000 X g for 10 minutes. The supernatant was filtered through syringe filtration, and then frozen overnight. The next day samples were thawed and 25 microliters of each sample were run through High Performance Liquid Chromatography (HPLC). Another sample from each flask was taken and the pH measured to be certain the bacteria were still alive.

HPLC

HPLC media was prepared regularly by mixing 570 microliters of concentrated sulfuric acid (H₂SO₄) in 2 liters of distilled water (diH₂O). 25 microliters of sample were loaded into an Aminex® HPX-87H Column for HPLC and run for a minimum of an hour. Each sample was run twice. Samples whose peak heights were significantly different were run a third time. The column was calibrated with varying concentrations of glycerol, acetic acid 1,3-PDO, ethanol, butyric acid, and butanol. The average retention time was determined, along with the slope of the line of the graph of concentration (g/L) vs. area under each peak (μ V*min) for each substance. This is recorded in Table 1.

Table1: Calibration of HPLC Machine				
Substance	Retention	Slope		
	time (min)	$(\mu V*min/g/L)$		
Glycerol	19.7	11444		
Acetic Acid	22.8	6790.1		
1,3-PDO	25.9	8227		
Ethanol	31.8	4593.5		
Butyric	33.5	9466.1		
Acid				
Butanol	55.5	8767.1		

Different Growth Conditions

Multiple flasks were used to determine the trends in glycerol consumption and butanol production. Variations included the size of the flask and the initial concentration of glycerol. The concentration of initial glycerol was doubled from 25g/L to 50g/L in two flasks to determine if the bacteria could survive at higher concentrations of glycerol. Two control flasks at 25g/L initial concentration were also grown.



Figure 1. Overview of metabolic pathways of *C. pasteurianum*. Dotted lines represent movement of electrons. R1 glycerol dehydratase, R2 PDO dehydrogenase, O1 glycerol dehydrogenase, O2 dihydroxyacetone kinase, 1 lactate dehydrogenase, 2 pyruvate–ferredoxin oxidoreductase, 3 hydrogenase, 4 acetaldehyde dehydrogenase, 5 ethanol dehydrogenase, 6 thiolase, 7 butyryl-CoA dehydrogenase, 8 butyraldehyde dehydrogenase, 9 butanol dehydrogenase The boxed area indicates the solventogenesis pathway of butanol formation. [1]





III. Results and Discussion

HPLC data was compiled for each flask tested. The calibration table was used to determine the concentration represented by each peak. An average concentration was determined for each sample. A chart was made of concentration of each substance over time. The charts for a 25g/L glycerol control and a 50g/L sample are included in Figure 2. These charts are a sample of the type of data collected.

No correlation was found between the size of the flask and the rates of butanol production. The size of the flask ranged from 100.0 ml to one liter. The only notable difference was that the cultures in larger flasks were sustained longer than those in larger flasks. This is primarily due to less space and less total resources available. However, the concentrations of butanol, glycerol, and other compounds were similar across all sizes of flask. As such, the focus of the data is more on the concentration of each substance and less on the total mass or volume.

It was found that in most cases butanol levels spiked between days 1 and 3 and then decreased steadily thereafter. This phenomenon is more easily observed in the 25B chart than the 50B chart. In the 50B sample, the butanol levels spiked on day one, decreased until day 3, and then spiked once on day 4 and disappeared entirely. More samples that were tested followed the trend of an initial spike followed by decreasing levels. The decreasing concentrations of butanol over time are believed to be due to its reabsorption back into the cellular membrane of dead bacteria cells [3]. Butanol, as with all alcohols, is known to be absorbed by the cell membrane thereby increasing membrane fluidity. Bacteria counteract this often toxic effect by altering the composition of their membranes and the ratios of unsaturated to saturated fatty acids. This response is known as the homeoviscous response [3]. If the butanol produced and excreted by the bacteria is reabsorbed over time, both by living and deceased cells, then the concentration of butanol would appear to go down, as our results show.

Based on this hypothesis, it is the intention of this lab group to test methods for removing butanol from solution. This will not only harvest the butanol, but also shift equilibrium towards butanol diffusing out of the cells. We intend to test pumping fresh glycerol media into the reactor and pumping used media out to replenish glycerol and remove butanol. Liposomes will also be used to remove butanol from the used media in order to harvest it. These methods are designed to aid continuous growth of a bacterial culture and prevent reabsorption of butanol by cells.

Overall, it was found that *C. pasteurianum* can grow at higher concentrations of glycerol than had been previously tested and produce butanol. The results of 50g/L glycerol were incredibly similar to those of 25g/L. Initial butanol concentrations were similar, within 0.1g/L of each other, and decreased at similar rates. However, butanol was only detected in one of the four flasks by day six. Despite the apparent lack of change in doubling the glycerol concentration, these results are promising. The bacteria is capable of surviving at higher concentrations of glycerol. Further testing of higher concentrations of glycerol may yield larger amounts of butanol produced and higher rates of production.

IV. Conclusion

Although our initial results do not show that an increase in glycerol concentration leads to an increase in butanol production, it is highly plausible that such an increase could lead to a higher rate of butanol production. Our theory that the butanol is reabsorbed by dead bacterial cells would account for the lack in apparent change in butanol levels from 25g/L of glycerol to 50g/L. It is possible that once this effect is compensated for, there will be a correlation between initial glycerol concentration and butanol production rate. At the very least, the preliminary results here have shown that the bacteria are capable of surviving at 50g/L glycerol. Further testing is necessary to determine if the rate of butanol production and the amount of butanol produced can be increased by increasing available glycerol. Pumping fresh media into the reactor and pumping old media out will be the next step in this project, followed shortly with the use of liposomes to remove butanol from the used media. Both of these processes should provide a more accurate way of measuring butanol production.

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Tokenism & Resistance:

Gender, Sexism, and Culture in the United States Military

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Abstract - Tokenism occurs when there are a small number of minority members within a work group. Token minority members sometimes experience structural and cultural practices which prevent them from advocating for one another or future minority members within the organization. Aspects of tokenism include heightened visibility, isolation, and stereotyping. This study uses interviews from female veterans in the United States military to understand their experiences of tokenism and how these experiences shaped their ability to resist sexism and advocate for cultural changes at work.

I. Introduction

Enlisting in the United States military can increase career opportunities and result in higher lifetime earnings (Padavic & Prokos, 2017). This is particularly true for members of socially and economically disadvantaged groups. Military service provides access to higher education through the GI bill and job training, and by providing a bridge between young adulthood and a career. Moreover, those who serve in the armed forces are more likely to later work in highly paid careers in science, technology, engineering, and math (Steidl & Werum, 2017). Additionally, military service helps integrate traditionally marginalized groups into the mainstream of American society (Fischer, Lundquist, & Vachon, 2016). This may be in part due to contact between majority and minority group members during their time of service, because increased contact between minority and majority groups can help break down perceived stereotypes over time. Another explanation may also be that those who share in the cost of maintaining society are entitled to an increased proportion of that society's representation and resources. Historically, one of the most successful arguments for including new elements as full members of American society has been predicated on military service during conflict (Salyer, 2004). One of the largest such previously excluded groups of American citizens are women.

Women benefit from serving in the armed forces. However, women currently comprise approximately 15% of the United States armed forces (Department of Defense, 2014). In 2016 women's opportunities for military service expanded as the Department of Defense opened almost every military occupational specialization (MOS) to female service members. More recently, the U.S. legislature has debated requiring women to enroll in selective service and studies are ongoing about the roles of women in the military.

The percentage of women in the armed forces has been slowly increasing (Patten & Parker, 2011). Women's opportunities within the military have been increasing, but integration has been slow and incremental. Relatively few women are enlisting and the reason why may lie in organizational culture. Adding a small number of women into a predominately male workplace may not be enough to break down barriers for full integration. This article will investigate how women experience their status as tokens in the armed forces and how these experiences limit women's ability to advocate for further gender equity.

II. Literature

In the 1970s, feminist scholars turned their critical lens on work and organizational cultures. One of the breakthrough theories developed in the course of this line of inquiry was Critical Mass Theory (Kanter, 1993). This theory was developed to understand why introducing a small number of minority members into an organization was insufficient to yield full integration. A token is a member of a small group within the larger group – in Kanter's work tokens were women in "skewed work groups" – where women were less than 15% of the total number of people in the group. Token minority members are unable to advocate for other minority members or create organizational transformations which would render the context more welcoming

because of their own token status within the organization (Kanter, 1993). More succinctly, there are not enough people to form a coalition to push for change.

Moreover, Critical Mass Theory outlines the mechanisms through which token minority members are deprived of opportunities and power within an organization. The organizational dynamics underlying these mechanisms include increased visibility, contrast, and assimilation (Kanter, 1993). Visibility manifests as a sense of being under increased scrutiny. Heightened visibility means that tokens feel pressure to perform at a higher level and fear sanctions for minor failures. Contrast leads majority group members to be more aware of the differences between themselves as a group within a group. According to Kanter, dominant group members may then make exaggerated displays about how intrusive they find token women. Loyalty tests were also part of the boundary heightening process. A loyalty test might be an off-color remark or a sexist joke and the token was expected to play along or not complain. Assimilation led to token minorities being more readily stereotyped - often resulting in women being expected to perform in gendered ways that would be inappropriate for their occupational status; for example asking female executives to perform secretarial tasks or expecting female employees to arrange meals or drinks at meetings. These mechanisms combine to continually remind token minority members that their position within an organization is precarious and that any demands or attempts at change will be met with resistance or reprisals.

There have been a variety of criticisms lodged against Critical Mass Theory. Notable among these is an acknowledgment that it does not account for inequality in the broader social context (Yoder, 1991; Williams, 1992). Those who are marginalized in society at large will be similarly disadvantaged in their workplace. For example, token men in majority female occupations are more likely to be promoted and do not experience the negative effects of tokenism. Individuals can also inhabit overlapping social categories which will produce qualitatively different experiences of accumulated social (dis)advantage (Collins & Bilge, 2016). Furthermore, the way that work organizations are structured has changed in recent decades. Workplaces rarely promote from within and that career trajectories are less well-defined (Williams, 2013). In light of these transformations, discourse about who is promoted from within and why may be less relevant.

Subsequently, the military is a unique workplace and an ideal test case to examine the validity and relevance of Critical Mass Theory. Firstly, the military is one of the few workplaces that still has a long career ladder with internal promotion. Secondly, more so than even in Kanter's case study, social life, residence, and occupational activities are bundled together within the military. Thirdly, the military is one example of a gendered organization. While the definition of a gendered organization can be symbolic and complex as it pertains to assumptions about who the ideal worker is or how work is carried out, one of the simplest definitions is that the majority of workers are male (Britton, 2000). The military qualifies on both counts. First, the majority of those in the military are men. Second, the common cultural perception of soldiers is that they are "physically and mentally tough, goal-oriented, aggressive soldiers with skills of violence, weaponry, and ultimately death" (Silva, 2008) and these attributes are commonly associated with masculinity. It has been argued that militarism is so deeply masculine that military service is part of the social construction of masculinity itself (Kimmel, 1996; Salyer, 2004). For these reasons the military is a worthwhile organization to examine to what extent Kanter's theory is still relevant.

In Kanter's work tokens' experiences decreased their potential for creating a more inclusive work place for future minority workers in their field or firm. Studies of Critical Mass Theory in other masculine work environments indicated that increasing the proportion of women was a baseline for institutional transformations but was not a guarantee of inclusion. Within the sciences, when women constituted less than 15% of a work group, men's behavior towards women was found to be chilly and exclusionary (Cain & Leahey, 2014). In the construction industry women have tended to split off into their own separate firms and committees. These majority female firms could advocate for change within the industry and institute more inclusive policies at the firm level-however, the industry as a whole remains stubbornly resistant to widespread integration and inclusivity (Greed, 2000). In medical practice, women in male dominated specialties have reported less supportive peer relationships (Wallace, 2014). Freedom from harassment and discrimination

were a baseline necessity for token members to advocate for more inclusive workplace policies. The present study will examine two questions. First, in what ways and to what extent did interviewees experience the hallmarks of tokenism? Second, did their experiences inhibit their self-perceived agency to advocate for a more generally inclusive workplace culture?

III. Data & Methods

The sample of interviews is a subset from a series of interviews collected for another project conducted by Dr. Christina Steidl at the University of Alabama in Huntsville which examined gender performativity and the military more broadly. This analysis draws on ten interviews by female veterans from across the United States. Time and feasibility constraints limited the number of interviews included for this project. Women in the sample were included the seven in the United States Army, two in the Navy, and one in the Air Force with a mix of active duty, Reserve, and National Guard represented. While over half of the participants spent some time in the Reserves or National Guard participants all served at least one year of active duty between 2005 and 2015. Consistent with the qualitative nature of the project this is a nonrandom purposive sample.

Dr. Christina Steidl recorded the interviews both in person and via Skype over a period of several months in 2015. The interviews were then transcribed by Brooke Killion, also of the University of Alabama in Huntsville. For the purpose of this analysis, the interviews were then read for common thematic patterns in the responses and analyzed with a grounded theory approach (Lofland, Snow, Anderson, & Lofland, 2006; Charmaz, 2006).

IV. Findings

Overall, participants reported that their experiences in the military were positive. The majority of interviewees entered the military for educational and occupational opportunities. Most found them. However, several common themes emerged during the interviews that are consistent with the mechanisms of social exclusion outlined by Kanter (1993). The first section of findings will describe women's experiences with visibility, isolation, and stereotyping, which are the behavioral manifestations of the exclusionary social processes. The second section of findings will describe interviewees' experiences of sexism in the workplace and how they did or did not respond. While the interviews were not focused on sexual harassment or discrimination, multiple interviewees voluntarily spoke about their experiences with these issues and their responses. Interviewees' descriptions of sexism and their responses give insight into how their experiences as tokens mitigated their ability to advocate for women collectively.

Tokenism & Exclusion

This section of findings will explore how female veterans experienced tokenism during their military service. Some of the women felt that failure was generalized and attributed to gender, while success was attributed to them as individuals. Thus, they felt increased pressure to perform better than their peers. While their status as exceptions sometimes worked to the women's advantage, they were often ambivalent about how benevolent sexism would impact their relationship with their co-workers over the long term.

Stacy described an incident where a male superior officer brought all the female soldiers hot chocolate on a cold day. While she appreciated the courtesy she expressed ambivalence about the attention and unequal treatment: "I'm not gonna you know, just be like, 'No, I don't want this...' but at the same time it's... it's so hard to like, that fine line. Like I want to be treated just like everybody else." Stacy also noted in her interview that male soldiers would remark about women getting special treatment – which justified her concerns about being singled out for special courtesies.

Kelly describes a situation where her gender made her more visible and more memorable and that she feared she would be judged by a different and more rigorous standard than her male colleagues: "*Like, they just, you didn't get away with anything, you know? And there was just this expectation that they just wait for you to mess up so they can drop the iron clad on you.*" Whether the result was positive or negative in the short term, those who felt that their token status made them more visible realized that they were not viewed the same as other soldiers. Some women in the sample felt isolated from informal social networks with their peers. When male workmates went out drinking, attended sporting events, or discussed hunting, women sometimes felt left out. One interviewee recounted how it made it difficult for her to bond with her male colleagues in the National Guard: "...*it's a little bit more macho. Slightly, I mean, yeah just a little bit. So, a lot of the guys were hunters, and they would sit around all day talking about bow hunting and I caught this and I shot this and I just didn't have you know, any way to relate to these guys.*" - Brenda

"A lot of the males were going out to a baseball game and they didn't even think to invite me. It was like 'Oh. Why do—I'd like to go!' and so sometimes it just takes a little bit of personal initiative to try to break down some of that stuff." – Harriet

Cultural mismatches like this sometimes occurred. While the women were reluctant to describe the process as deliberate exclusion, the result meant that the women missed opportunities to connect with coworkers or exchange information about work during informal social events. The lack of women coworkers made some women feel lonely or isolated. Ingrid closed her interview with the summation, "*I guess my closing comment would be to say that being a female in the military is a very lonely*" because there are so few women to interact with.

Many of the women spoke about the hostile stereotypes they encountered. The primary stereotypes were that women were lazy, promiscuous, or incompetent, as illustrated by the following quotation: For example, Rhia said, "for females, a lot of the negative comments that I got were that they didn't feel that she was physically capable." Three of the women had similar experiences of stereotyping, with a common worry from their male colleagues that they would be a burden because of laziness, weakness, or promiscuity.

Some stereotypes were more subtle – women sometimes described being asked to do things which were outside of their normal work responsibilities – such as handling planning for social events, acting as a secretary, or doing extra cleaning because they were women. These interactions and stereotypes were an omnipresent reminder to women tokens that they did not belong and would not be seen as equal unless they worked harder to prove themselves. Even when women fought for and gained acceptance, these women viewed their status as precarious. The price for admission into the boy's club was steep.

Acceptance & Resistance

This section will elaborate on how the women's experiences as tokens prevented them from changing the culture and structure that might harm other female soldiers. There is already a robust set of scholarship and popular discourse about sexism and harassment in the military, therefore, none of the interview questions were intended to prompt interviewees to discuss these issues. However, it became clear during the research process that sexism shaped women's daily lives. While relatively few women felt they had been in danger many spoke with resignation about incidents which could be viewed as demeaning or sexist in other workplaces. Sexist interactions could be included within the framework of tokenism-women's acceptance of workplace sexism was shaped by their precarious inclusion in their work groups.

Examples of women accepting sexism occurred in every interview where women discussed sexual harassment and sexist behavior. For example, Erin said, "if you're in a locker room, it's a guy's locker room, you're going to hear stuff. And you've just got to let it roll of the back. Don't take everything seriously... I just think that if you realize it's more of a guy's world than a girl's." Another interviewee, Quianna said, "'Hey, if you don't like it then go somewhere else,' type of deal. You know, versus if a male – you know, and I feel like I'm sounding very biased but at the same time it's just really kind of like the reality. It's the reality." combined illustrate These quotes women's acquiescence to sexism as being the price of acceptance and success in the military.

The women's reports of sexism ranged from crude remarks framed as jokes, to staring, to quid pro quo sexual harassment. Their responses never included a single official complaint to their chain of command. There are formal policies in place for reporting discrimination and sexual harassment in the military, yet women in this sample did not use these formal channels of resistance. Nor did the women use informal channels to resist discrimination, such as directly challenging colleagues or superiors who participated in sexist discourse. Instead, it was understood that a certain level of sexism was to be tolerated and that the onus was on individual women to protect themselves from unwanted sexualization, even when circumstances made it impossible for women to implement the anti-harassment guidelines that were outlined for them. Melissa recalled seeing a large poster about how women could avoid sexual assault and noted how her billeting during deployment did not allow her to follow any of the safety guidelines: "Women should only walk in well-lit areas. Okay, well you billeted me on the other side, women should be billeted together, women should be moving—I literally could do none of the things on the list."

This response illustrates that women understood that they were responsible for enforcing boundaries about appropriate sexual conduct. Combined with their concerns about being rejected for overreacting, complaining, or being accused of falsely reporting harassment, their relative isolation made collective advocacy for change exceedingly difficult. Still, women did manage the situation sufficiently in most cases to their own satisfaction. Some women asserted that their male colleagues' behavior had, at some point, crossed a line and was inappropriate. In the words of Allison, "I project myself as you know, and I apologize for the word, but as someone who's not going to take any shit from people." Others, like Vanessa, regretted that they did not feel empowered to push back: "...but I don't in the military, and I think I actually don't act that way because of the stereotypes that are with that and because I know we're a giant minority. And I don't say a lot of things to act against or anything like that." The interviewees proved to themselves and their fellow soldiers time and again that they were capable, fit, and qualified. However, proving their status as soldiers by not objecting to routine sexist interactions came at a cost. Individuals secured their tenuous acceptance in part by acquiescing to the status quo.

V. Discussion & Conclusion

These interviews reveal an important factor of tokenism and resistance; numbers matter. Women in the military do sometimes still experience the impacts of being a minority within a majority: visibility, isolation, and stereotypes are constant reminders that they are not going to be immediately and unquestioningly accepted. Although the struggle to find acceptance is not insurmountable, women understand that their position is insecure and their connections are more tenuous. In sum, forty years after the initial publication, Kanter's Critical Mass Theory is still relevant

On a practical level this means that when women face sexism at work in the military, they have fewer options for resisting. Most of the women found individual solutions. They managed to protect themselves and disarm the hostility of their fellow soldiers. The women were generally uncritical and fatalistic about the masculine culture of the military and their own position within it. In the words of Brenda: "It is what it is." Furthermore, all ten of the women in the sample attributed their experiences with harassment and sexism as the action of a few bad actors rather than a systemic issue even though all of them could recall at least one incident of harassment, sexism or discrimination. Four of the women in the sample used phrases like "locker room culture" and one, Vanessa, specifically said that she could not express empowerment because "Every once in a while I kind of get a hint of something like, 'Go women power!' but I don't in the military, and I think I actually don't act that way because of the stereotypes that are with that and because I know we're a giant minority."

Women individualized and trivialized their experiences with sexism and their responses to sexism. Perhaps this was because there were few other women around to socialize with. Even when other women were present, the code of silent acceptance was strong. Regardless, the lack of other women and the contingent acceptance among men meant that there was little groundwork for collective resistance and cultural change. The prospects for individual women are positive, but women are not structurally or culturally positioned to open a path for further integration.

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There is no evidence for bone remodeling caused by transdermal calcium loss in sweat during Bikram Hot Yoga in premenopausal women

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Abstract – It has been hypothesized that sweat loss during exercise causes a disruption in calcium homeostasis leading to bone resorption and low bone mineral density. There is substantial water and electrolyte loss in sweat during hot yoga, an exercise that is becoming increasingly popular with premenopausal women. A Bikram Hot Yoga session typically consists of 26 Hatha Yoga postures that are performed in 90 minutes inside of a room that is set to 105 °F and 40 % humidity. We measured sweat and plasma, electrolyte and water balance before and after a hot yoga session in a population of premenopausal women. Sweat was collected during the final yoga asana by saturating filter paper with sweat from the participants' thighs to estimate the total amount of electrolyte loss. There was no change in serum sodium or serum osmolarity before or after Bikram Hot Yoga. Mean calcium concentration in serum increased after the hot yoga session. The concentration of parathyroid hormone (PTH) did not change from before to after the hot yoga session; however, a substantial amount of water was lost. This implies that the amount of parathyroid hormone in circulation decreased from before to after. Calcium loss in sweat loss did not trigger an increase in PTH secretion and did not initiate bone resorption. A disruption in calcium homeostasis was not observed in a bout of excessive sweating during a 90-minute Bikram Hot Yoga session. Bikram yoga appears to be a safe practice for premenopausal women as long as fluid and electrolytes are appropriately replenished after the Bikram Hot Yoga Session.

I. Introduction

Bikram Yoga is a method of Hatha Yoga created by Bikram Choudhury and is classified as hot yoga (Choudhury, 2007). Bikram Yoga is a sequence of 26 Hatha Yoga asana and two breathing exercises performed at 105 °F and 40 % humidity. Each posture

is repeated twice in a class lasting 90 minutes. There is significant sweat, water, and electrolyte loss due to the high temperature in a Bikram Yoga studio. Hot yoga is a non-loading exercise that is becoming increasingly popular with pre-menopausal women. Several studies have pointed towards many health benefits from practicing hot yoga, including increased strength and flexibility (Hewett et al. 2015; Field 2016), weight management (Pate & Buono, 2014), and improves arterial stiffness (Hunter et al. 2016). However, it has been hypothesized that large losses of calcium in sweat during exercise causes a disruption in calcium homeostasis, which may lead to bone resorption and low bone mineral density. Any potential risk for decreasing bone mineral density should not be taken lightly, as this population of women is at a higher risk of developing osteoporosis.

A large calcium loss in sweat would elicit a decrease in serum calcium concentration that would be detected by the parathyroid glands. In response, they will produce and release parathyroid hormone (PTH). PTH stimulates osteoclasts to break down bones, releasing calcium back into the extracellular fluid compartment. PTH also has effects on the kidneys and downstream effects on the gastrointestinal tract; it causes them to reabsorb and absorb more calcium, respectively. All of these processes function to increase the serum concentration of calcium (Fig. 1). Once serum calcium concentration reaches an appropriate level, negative feedback inhibition will prevent more PTH from being produced.

There is evidence to suggest that excessive dermal calcium loss in sweat leads to bone mineral content (BMC) decreases in young male athletes, with around 422 mg of calcium lost in sweat per basketball game. This leads to a 10.5% decrease in the BMC of their legs over the course of a basketball season, thus increasing the risk for bone breaks and fractures (Klesges et al. 1996). A study in female competitive cyclists showed that physical-exercise induced calcium loss in sweat was associated with an increase in biomarkers of bone metabolism, such as PTH and CTX-I (Haakonssen et al. 2015). The increase in PTH during exercise could be attenuated by a pre-exercise high calcium meal (Barry et al. 2011). It was observed in athletes with similar training regimes that lower BMC was associated with an increased risk of stress fractures. Interestingly, these athletes also had a lower dietary calcium intake and menstrual irregularities (Myburgh et al. 1990).

Premenopausal women athletes may be at a much higher risk of bone fracture due to increased loss of calcium in sweat during exercise. Premenopausal women generally show a loss in bone mineral density of 0.3-1.3% per year at the femoral neck and lumbar spine, respectively, caused by hormonal changes that alter calcium levels and decrease BMC (Bainbridge et al 2002). Additional losses of calcium in sweat caused by multiple sessions of hot yoga per week may decrease the BMC and increase the risk for bone fractures unless accounted for by increased intake of calcium in the diet.

The purpose of this study was to measure the transdermal calcium loss from thermal sweating in premenopausal women participating in Bikram Hot Yoga. We will determine if this elicits a physiological response by measuring changes in PTH, a biochemical marker of bone remodeling.



Figure 1. A schematic representation of the homeostatic response to low blood calcium.

Any excessive calcium loss in the sweat, urine, or feces would lower blood calcium in the extracellular fluid (ECF) from the normal range of 8.9-10.1 mg/dL. This is sensed by the calcium sensing receptor expressed in the parathyroid glands (yellow ovals) on the posterior side of the thyroid gland. They release parathyroid hormone (PTH) into the blood which acts on the kidneys to increase reabsorption of calcium from the urine, and on the gastrointestinal system to increase calcium absorption. In addition, the kidneys also release calcitriol, also known as 1,25(OH)2D or Vitamin D, which increases absorption of calcium in the gastrointestinal tract. The most rapid effect of PTH is to stimulate bone osteoclasts to break down bone (resorption) and release calcium into the ECF increasing blood calcium concentrations. This can be detected by the calcium sensing receptor and result in negative feedback and decrease PTH secretion.



Figure 2. The 26 Asana of Bikram Yoga.

1. Half Moon (Arda-Chandrasuna). 2. Hands to Feet (Pada-Hasthasana). 3. Awkward Pose (Utkatasana). 4. Eagle Pose (Garurasana). 5. Standing Head to Knee (Dandayamana-Janushirasana). 6. Standing Bow Pose (Dandayamana-Dhanurasana) 7. Balancing Stick (Tuladandasana) 8. Standing Separate Leg Stretching Pose (Dandayamana-Bibhaktapada-Paschimotthanasana). 9. Triangle Pose (Trikanasana). 10. Standing Separate Leg Head to Knee Pose (Dandayamana-Bibhaktapada-Janushirasana). 11. Tree Pose (Tadasana). 12. Toe Stand (Padangustasana). 13. Dead Body Pose (Savasana). 14. Wind-Removing Pose (Pavanamuktasana). 15. Sit-Up. 16. Cobra Pose (Bhujangasana). 17. Locust Pose (Salabhasana). 18. Full Locust Pose (Poorna-Salabhasana). 19. Bow Pose (Dhanurasana). 20. Fixed Firm Pose (Supta-Vajrasana). 21. Half Tortoise Pose (Ardha-Kurmasana). 22. Camel Pose (Ustrasana). 23. Rabbit Pose (Sasangasana). 24. Head to knee Pose and Stretching Pose (Janushirasana and Paschimotthanasana). 25. Spine-Twisting Pose (Ardha-Matsyendrasana). 26. Blowing in Firm Pose (Kapalbhati in Vajrasana). All postures are performed twice in a room heated to 105 °F at 40 % humidity over 90 minutes.

II. Materials and Methods

Study Population

Participants were female yogis selected by a flyer posted in the Bikram Hot Yoga of Huntsville studio (Madison, Al). A total of 5 subjects were recruited for this small viability study. Subjects with kidney disease, who use any medication known to affect bone metabolism, who are post-menopausal, and who use blood thinners were excluded from the study. Participants' mean age was 47.4 ± 4.7 years (n = 5). The subjects had attended a mean of 3.8 ± 1.4 Bikram yoga sessions per week for the last 2.0 ± 1.8 years. Sweat, pre and post yoga blood samples were collected from all 5 participants. This study was approved by the University of Alabama in Huntsville Human Institutional Review Board.

Study Measurements

Nude body weight was measured before and after the Bikram hot yoga session (Withings Body Scales, Withings, Inc., Cambridge, MA). Each participant's water bottle was weighed before and after the yoga session using a Philips Hipster Coffee Scale (Philip Ambrose, Five Points, Huntsville, AL) to calculate water volume consumed during a hot yoga session. Sweat was collected using a 5 cm x 5 cm square of filter paper (Whatman, No. 3) that was pressed against the thigh during the final Savasana posture. The thigh skin region was a good single representation of the whole body sweat sodium and chloride concentrations (Patterson et al. 2000). A blood sample was collected from all 5 participants immediately before and immediately after the 90minute session of Bikram hot yoga.

Serum and Sweat Analysis

Blood was collected in a serum separator tube and allowed to clot. Serum was collected by centrifuging the blood at 1500 X g for 5 minutes at 4 °C. The serum was then aliquoted for analysis. Sweat was collected by centrifuging the sweat-loaded filter paper in a Salivette tube at 2500 x g for 2 minutes and was frozen for analysis (Sarstedt AG & Co., Nümbrecht, Germany).

Serum and sweat calcium concentration were measured using a Calcium Colorimetric Assay Kit Cat. No. K380 (BioVision Inc., Milpitas, CA). Serum sodium was measured using a colorimetric sodium assay Catalog No. DZ114B-K (Diazyme Laboratories, Inc., Poway, CA). Serum PTH was measured using enzyme-linked immunoassay Catalog No. EIA-PTH (RayBiotech, Inc, Norcross, GA). Serum osmolarity was measured using a Micro-Osmometer Model 3300 (Advanced Instruments, Inc, Norwood, MA). Sweat sodium chloride concentration was calculated using a sweat conductivity analyzer Model 3120 (Wescor Inc, Logan, UT). In all cases manufacturer's instructions were followed for assays.

Body Volume Fluid Shift Calculations

For body volume fluid shift calculations total body water was assumed to be 50 % of body weight. The intracellular fluid compartment (ICF) was estimated to be 60% of total body water and extracellular fluid compartment (ECF) was estimated to be 40 % of total body water. Plasma volume was estimated to be 25% of the ECF. Theoretical changes in plasma sodium, calcium, and PTH levels were calculated using the water, sodium and calcium losses measured in sweat. The general assumptions were that intracellular solutes did not shift between the ECF and ICF compartments and that all electrolyte and fluid losses came from the extracellular fluid compartment.

Statistical Analysis

All data except PTH measurements are presented as mean \pm SD and were compared using a paired two-tailed Student's t test with a P value of P < 0.05 being considered as statistically significant. PTH measurements were presented in the same manner; however, a one-tailed Student's t test was performed with a P value of P < 0.05 being considered as statistically significant. All of the data was plotted and statistically analyzed using Prism 6.0 software. All assays were performed in triplicate.

III. Results

Nude body weight was initially 60.4 ± 5.5 kg and was 59.2 ± 5.4 kg (n = 5) after 90 minutes of Bikram Hot Yoga. A decrease of 1.2 ± 0.6 kg resulted in a decrease of 1.92 ± 0.90 % body weight. During the 90 minutes of hot yoga the yogis drank an average of 0.38 ± 0.22 (n = 5) liters of water. Adjusting for the amount of water drank during the 90 minute yoga session and assuming that all body weight loss was due to sweat production, the total sweat production during the 90 minute session was calculated as 1.54 ± 0.65 liters (n = 5). The volume of sweat lost and water consumed for all participants is shown in Figure 3.

First, we looked at body water and salt homeostasis. Serum osmolarity (Fig. 4A) was 276.4 \pm 4.8 mOsm/L before yoga and 284.3 \pm 7.6 mOsm/L after yoga (P = 0.2089, n = 5). Serum sodium (Fig. 4B) also did not change and was 137.5 \pm 7.7 mmol/L before and 138.0 \pm 5.4 mmol/L (P = 0.9188, n = 5) after hot yoga. Although 1.54 \pm 0.65 liters (n = 5) of water (Fig. 3), and 164 \pm 32 mmol/L of sodium chloride were lost in sweat, there was no change in body serum osmolarity and serum sodium concentration.

Plasma calcium concentration (Fig. 4C) increased from 10.4 ± 0.8 mg/dL before to 11.3 ± 0.8 mg/dL (P = 0.0017, n = 5) after hot yoga. There was no change in parathyroid hormone concentration, a marker of bone reabsorption (Fig. 4D). Serum PTH did not change and was 33.9 ± 3.3 pg/mL before hot yoga and 33.5 ± 3.3 pg/mL (P = 0.6609, n = 5) after hot yoga. The total calcium lost in sweat during the 90 minute period was estimated to be 41.3 ± 16.4 mg (n = 5).

In order to calculate changes in plasma volume, we used theoretical body water compartment calculations to determine if any of these changes could be explained by a decrease in plasma volume. The theoretical ECF concentration for calcium using the observed sweat loss and calcium lost in sweat was 11.6 \pm 1.2 mg/dL. The theoretical measurement was not statistically different from the observed measurement $11.3 \pm 0.8 \text{ mg/dL}$ (P = 0.3134, n = 5); however, it was 10.1 % higher than the measured pre-yoga serum calcium concentration which was 10.4 ± 0.8 mg/dL (P = 0.0206, n = 5). This finding suggests that the observed increase in serum calcium concentration after hot yoga can be explained by the amount of water lost in sweat and the corresponding decrease in plasma volume.

The theoretical concentration of PTH was 36.4 ± 4.0 pg/mL after plasma volume reduction from sweating, whereas measured PTH concentration was only 33.5 ± 3.3 pg/mL. This implies that the amount of PTH in circulation decreased by about 15% (P = 0.0251).



Figure 3. Fluid balance in yogis performing 90 minutes of Bikram Hot Yoga.

During the 90 minutes hot yoga session, the yogis lost 1.54 ± 0.65 liters (n = 5) in sweat. This loss was not completely replaced as the yogis drank only 0.38 ± 0.22 liters (n = 5) of water.



Figure 4. Measurement of electrolytes, osmolarity and PTH before and after Bikram Hot Yoga.

There was no difference in serum osmolarity (A) and serum sodium concentration (B) before or after yoga practice. However, serum calcium increased from 10.4 \pm 0.8 mg/dL before to 11.3 \pm 0.8 mg/dL (P = 0.0017, n = 5). There was no change in serum PTH concentration before and after a hot yoga session.

IV. Discussion

The primary goal of this study was to quantify the transdermal loss of calcium from sweat and measure potential markers of bone resorption during extracellular fluid calcium homeostasis. There is a large discrepancy (approximately 1.2 liters) between the volume of sweat produced and volume of water consumed during a 90 minute practice of Bikram Hot Yoga. There is also a large quantity of sodium and a moderate amount of calcium lost in sweat. This raises the possibility of an electrolyte imbalance causing bone resorption to maintain the serum calcium levels at normal values. However, there was about 40 mg of calcium lost in the sweat. This small amount of calcium, in combination with the large amount of water lost can explain the increase in serum calcium concentration. Surprisingly, our data showed no change in serum sodium concentration or osmolarity before and after 90 minutes of hot yoga. This was due to the large amount of sodium lost in the thermal sweat during yoga.

PTH levels increase in response to low blood calcium levels. An increase in PTH triggers bone resorption, an increase in kidney calcium reabsorption, and an increase in intestinal calcium absorption. All of these mechanisms cooperate to increase extracellular fluid compartment calcium concentrations (Fig. 1). In our study, serum calcium concentration increased after hot yoga which would not cause an increase in PTH secretion from the parathyroid glands (Fig. 5). Furthermore, it was shown that the amount of PTH in circulation decreased.

Transdermal calcium loss from sweat leads to an increase in serum PTH levels and is associated with decreases in bone mineral density (BMD) in cyclists (Sherk et al. 2014), but not in yoga (Lu et al. 2016). This may be due to the effects of weight-bearing exercise on bones. Cycling is not considered a weightbearing exercise and does not stimulate bone growth while yoga is a body weight-bearing exercise and may cause bone remodeling resulting in an increase in BMD. In a recent study 12 minutes of Hatha Yoga daily for 2 years resulted in an increase in BMD of the spine and femur and reversed osteoporotic bone loss (Lu et al. 2016). The release of PTH by modulators other than serum ionized calcium is poorly understood. Increased phosphate release from muscle during intense exercise has been shown to stimulate PTH secretion (Townsend et al. 2016). There is no information available on the effect of other byproducts of moderate muscle usage, such as increased carbon dioxide, serum lactate levels, decreased pH, and increased temperature have on the release of PTH from the parathyroid glands.



Figure 5. Model of water, salt, and calcium loss during 90 minutes of Bikram Hot Yoga.

In this schematic, it is shown that the water consumed was insufficient to replace the total amount of water lost in the sweat. Roughly 1.2 liters of water was lost. Due to the decrease in volume of extracellular fluid compartment, serum calcium concentration increased from before to after hot yoga, which was sensed by the parathyroid gland. In response, PTH secretion decreased, which implies that no bone remodeling occurred to replace the moderate amount of calcium lost in sweat. A large amount of sodium was lost in the sweat. Serum sodium concentration remained the same before and after the hot yoga session.

Our findings show that there is no evidence that the moderate amount of calcium lost in the sweat during Bikram Hot Yoga decreases serum calcium levels and initiates bone resorption thought release of PTH. Surprisingly, the decrease in plasma volume, together with moderate calcium losses in the sweat increases the apparent calcium concentration in the circulation, and produces a decrease in PTH secretion from the parathyroid gland (Fig. 1 & Fig. 5). This would set the necessary conditions for bone growth, although we would need conclusive evidence from BMD measurements to make this statement for a certainty. Future work includes measuring BMD, other minerals, and electrolytes lost in sweat during yoga and other bone-loading exercises.

In summary, despite the loss of calcium in sweat during yoga, there is no evidence that Bikram Hot Yoga stimulates bone loss to maintain extracellular fluid calcium levels. Bikram yoga appears to be a safe practice for premenopausal women. Our data does suggest that a large amount of fluid and sodium is lost, and a moderate amount of calcium is lost, and these should be replenished when rehydrating throughout and after hot yoga practice.

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