WEBER STATE UNIVERSITY

ERGO

UNDERGRADUATE RESEARCH JOURNAL

ERGO

er•go \er-gō\ adv. [latin] consequently, therefore; prep. on account of

CONTENTS

- v Journal Staff
- vi Research Reviewers
- vii Acknowledgments
- viii Letter from the Editor
- ix Introduction by Stephen A. Wise

FEATURED ARTICLES

- I Genderlect and the MBTI: Creating Social Coding Theory James Elmer
- 9 A Psychological Analysis of College Basketball Players James Bokinskie
- 14 Effects of Hemolysis and Lipemia on Immunoassay Results Van Aston & Aaron Harper
- 21 The Human Metapneumovirus in Pediatric Patients of Utah Clinton Potter & Devin Christensen
- 27 Comparison of FDA-Approved Antibiotics to Non-Prescription Mexican Antibiotics Trina Riley, Leah Daily & Tyler Roe
- 38 The Effects of Stress on Platelet Function Alisha Weiss, Nathan Fenn & Majak John
- 45 Isolation and Characterization of Chitin-Utilizing Halophiles from the Great Salt Lake, Utah

 Kevin M. Bowcutt, Brigham Burton & Dan R. Cox

iv ◆	Contents
57	Inhibition of Common Spoilage Fungi by Lactic Acid Bacteria Karli E. Oberg, Miriam N. Bernardo & Kris Froerer
68	Hold 'em or Fold 'em: Hindlimb Flight Posture of Utah Shorebirds Lani S. Shepard & Joshua C. McFarland
79	Rejecting Huntington's Global Abridgement: Testing the Clash with an Analysis of Identity in Taiwan Zachary Vono
86	Did You Teach Them to Say Thank You? Teaching and Evaluating a Social Skills Curriculum Ronald W. Partridge, Lori Lundell & Shelly Pace
94	Extraction and Analysis of <i>Trans</i> -fat in Girl Scout Cookies Michael Campbell
RESE	ARCH ABSTRACTS
101	College of Arts and Humanities
102	Dr. Ezekiel R. Dumke College of Health Professions
111	College of Science
120	College of Social and Behavioral Sciences

126

Interdisciplinary Programs

JOURNAL STAFF

Editor-in-Chief

Samantha Balaich

Assistant Editors

Christopher Bentley Beau James Burgess

College Liaisons

Beau James Burgess

Nathan A. Canova

Lucas K. Hall

Rachel Johnson

Lori Lundell

David L. Wood

Layout and Design

Tyler Whitby

Faculty and Staff Advisors

John F. Cavitt: Director, Office of Undergraduate Research

Kathy Payne: Associate Professor, Stewart Library

Emily Stanger: Office Manager, Office of Undergraduate Research

RESEARCH REVIEWERS

Student Reviewers

Rachel Johnson Elly Alvarado Dave Baker Jessica Klem Samantha Balaich Lori Lundell Christopher Bentley George Mosier Jeanette Broadbent Christy O'Neil Beau James Burgess Shelly Pace Evan Carlisle Kyle Peterson Mike Cranney Blake Rabe Lucas K. Hall Debra Schiffman Amanda Johnson Ami Uchida Janet Johnson Melissa Ward Kevin Johnson David L. Wood

Faculty Reviewers

Daniel Bedford Michell Oki Shelly Morgan Costley Michael Olpin Matthew J. Domek Judith Pratt Nancy Haanstand Travis M. Price. Jim Hutchins Russell Rampton Jim Jacobs Rob Reynolds Gary Johnson Kent Ripplinger Thom Kuehls Yasmen Simonian Sheila Leali Joan Thompson John Mull Michele D. Zwolinski

ACKNOWLEDGMENTS

Each article published in *Ergo* was peer reviewed by a committee of students and faculty from Weber State University, and all of the research abstracts were presented at state, regional, or national conferences by the students who developed them.

The staff and faculty advisors of *Ergo* would like to thank the students and faculty members who volunteered their time and expertise to make *Ergo* a better journal as well as many others who were willing to contribute. The success of this journal in future years will depend on the continued involvement and interest of every college and department at Weber State.

The Office of Undergraduate Research would also like to sincerely thank the following who have contributed to the undergraduate research efforts at Weber State University over the past year:

Ralph Nye Charitable Foundation

Gloria Z. Wurst

LETTER FROM THE EDITOR

Introduction

"Somewhere, something incredible is waiting to be known."

Carl Sagan, American astronomer

Discovery is the echo from the world heard by those who have dared to push curiosity to the extreme. It is the triumphant sigh of relief after years of sacrifice and searching. It is the sudden idea that awakens the researcher in the middle of the night, and it is the undeniable result of undergraduate research done at Weber State University.

Discovery is brilliantly displayed throughout the pages of this journal. The submissions selected for publication in this edition of *Ergo* take readers from inside the minds of college basketball players to soaring aloft with select species of birds. Each published piece featured within these pages is only an inkling of the hard work, determination, and dedication to excellence that has been put forth by each student and faculty mentor. In addition, this year, we are excited to include an introduction from Stephen A. Wise, Ph.D., a WSU alumnus who has remained heavily involved in research since his undergraduate career at Weber State.

This second edition of *Ergo* would not have been possible without the amazing collaborative efforts of my staff. Each member of our team brought expertise from diverse areas of campus, allowing *Ergo* to continue its legacy of displaying undergraduate research efforts from a wide variety of backgrounds and disciplines. Their hard work and dedication to undergraduate research have made the journal you now hold a tangible reality.

Samantha Balaich Editor-in-Chief It is a pleasure to provide this introduction letter for the 2008 issue of the Weber State University undergraduate research journal *Ergo*. I graduated from Weber State University (then Weber State College) in 1972 with a B.A. in Chemistry and went on to receive a Ph.D. in Analytical Chemistry from Arizona State University. When I arrived at graduate school, I found that my chemistry undergraduate education at WSU had prepared me far better than most of the other students coming from larger universities. As part of my undergraduate research experience at WSU, I was introduced to the technique of gas chromatography, and I became fascinated with the power of chromatography. My exposure to chromatography through this undergraduate research opportunity directly influenced my selection of a graduate school and my specific field of study, my choice for my first position as a research chemist, and ultimately my 30+ year career as an analytical chemist at the National Institute of Standards and Technology (NIST).

In my current position as head of the Analytical Chemistry Division at NIST, I have the opportunity of leading approximately 100 scientists involved in research focused on developing and improving analytical methods and standards for the determination of chemical species in support of environmental, health/clinical, food, nutrition, forensic, and fuel/commodities measurements. Even though I am no longer actively "in the laboratory," I still consider myself a "chromatographer" and take pride in the excellent chromatography research performed by my colleagues at NIST. The scientists in the NIST Analytical Chemistry Division (approximately 80% have a Ph.D.) come from universities and colleges across the country, and the majority received their undergraduate education at small undergraduate only universities/colleges such as WSU. Almost all of our current postdoctoral research associates come with research experience gained in undergraduate research projects, not only at their university or college, but also from industry or government laboratories.

At NIST the value of undergraduate research experience is recognized. Each summer approximately 80 undergraduate students from across the country participate in the NIST Summer Undergraduate Research Fellowship (SURF) program. In the Analytical Chemistry Division, we typically have 6-8 undergraduate students working during the summer with our scientists on research projects. Many of these undergraduate students produce research results worthy of publication in peer-reviewed journals.

I commend WSU for providing undergraduate students with the opportunity to publish their research in a journal such as *Ergo*. The experience of preparing research results for a peer-reviewed publication is an essential part of academic training. I remember my feelings of excitement and pride as a graduate student when my first research paper appeared in a scientific journal, and I still have similar feelings of excitement even now when my name appears on a published research paper.

I hope that the research publications featured in this issue of *Ergo* will serve as a catalyst to the student authors to continue and to expand their research experiences.

Stephen A. Wise, Ph.D.

Weber State University, Class of 1972

Chief, Analytical Chemistry Division

National Institute of Standards and Technology (NIST)

Gaithersburg, Maryland

FEATURED ARTICLES

Genderlect and the MBTI: Creating Social Coding Theory

James Elmer*

Faculty Mentor: Colleen Garside

Abstract

Deborah Tannen's "Genderlect" theory did not address the impact of cognitive style, called "attitudes" by the Myer's Briggs Type Indicator (MBTI). The purpose of this study is to pilot test a theory that addresses the above-mentioned shortcoming. Social Coding Theory (SCT) seeks to find a causal link between an individual's need for connection and status (referred to as "social needs" in SCT) and the individual's cognitive style - introversion or extroversion (referred to as "social preference" in SCT). Balancing these two scales together identifies four personality types (referred to as "social codes" in SCT). SCT matches their "social code" to four outlined leadership styles (analyst, expresser, director, and influencer). The survey involved fifty participants at a western United States undergraduate institution. Analysis of the pilot study found a strong relationship between the individual's social preference and his or her leadership style. The strength of the findings between the social preference and the leadership style provides justification to conduct the study on a larger scale.

Introduction

In the early 1940's, Katherine Briggs and Isabel Myers developed what would come to be known as "Myers-Briggs Type Indicator" (MBTI), based upon Carl Jung's work on persoanlity typing. Social Coding Theory adds to their theory by asking two questions:

- 1. Is there more than one type of introvert or extrovert?
- 2. If so, what determines the various different types?

The main focus of this study was to look for a correlation between social preference (introversion or extroversion) and social need (need

for social connection and/or status). This study was also designed to analyze Deborah Tannen's theory of "Genderlect," which states that masculine individuals communicate for status while feminine individuals communicate for connection through what Burke calls "identification": attempting to act, believe and talk like the other party(ies) in the relationship (Larson, 2004, p. 11).

Overview

In 1997, the term "social codes" was coined to refer to assigned social labels where "virtually everybody...knows what they are and [agrees] they count in social relations" (Ridgeway, p. 3). According to Ridgeway, "the structural conditions of society...shape who encounters whom in society" and those structural conditions "frame what is likely to happen in those encounters" (Ridgeway, p. 9). Since a society is structured by people, the structural conditions of that society would depend upon the structural conditions of the people: their "social codes" (Ridgeway, 1997, p. 3). Tannen assumes the interpersonal need for connection and status stems from the femininity or masculinity of an individuals' communication style. SCT, on the other hand, assumes those needs stem from the cognitive preference (introversion or extroversion), referred to here as "social preference." Every individual has social need for connection and status. By creating scales for social need and the social preference and combining the two together, we can determine an individuals' "social code," as we see in Table 1.

 Table 1
 Social Coding Grid.

	Low Status Need	High Status Need	
Low Social Need	Comfortable Introvert (1)	Uncomfortable Introvert (2)	
High Social Need	Uncomfortable Extrovert (3)	Comfortable Extrovert (4)	

- A *comfortable introvert* prefers to stay at home and has little to no need for connection or status.
- An *uncomfortable introvert* is comfortable being home alone on a Saturday night but does not prefer to do so.

^{*} WSUSA Undergraduate Research Scholarship Travel Award

- An *uncomfortable extrovert* would more likely prefer to be invited to the party but enjoys attending nonetheless. He or she might host a party if the conditions are right.
- A *comfortable extrovert* may plan in advance to make sure they know where the party(ies) is/are and will feel comfortable crashing the party. He or she is most likely of all to host a party.

SCT does not argue that a person's social code is fixed, but just the contrary. Social codes are in a constant flux based on the social experiences of the individual and the context of the environment the individual finds him or herself in.

The Three Tenets

Social Coding Theory stands by three tenets:

- There are two cognitive styles: introversion or extroversion, (social preference),
- Every individual needs connection and status (social need), which is met via interpersonal communication and
- 3. Social Preference (SP) + Social Need (SN) = "Social Code" (SC).

Image Maintenance

"The forming, maintaining and enhancing of relationships with other people is one of the most powerful drives for people to engage in communication" (Van Baren et al., 2001, p. 14).

One of the reasons individuals seek for social connection and status in a relationship is to create and maintain an image that will validate their position in the relationship. People seek image maintenance for two reasons:

- 1. avoidance of inclusion-threatening situations and
- 2. response to exclusion warnings (MacDonald & Leary, 2005, p. 204).

An inclusion-threatening situation is an initial warning (e.g. what

a parent will say to a misbehaving child in the back of the vehicle), whereas the response to exclusion warnings will be the child's behavioral response.

Inclusion Threatening Situations

In inclusion-threatening situations, the individual percieves a possible exclusion from his or her social group resulting from what Alexander (1986) called a "status shift" (p. 117), which includes the use of language to reframe an individual in a way that promotes exclusion from the relationship. The shift in social status prompts a conforming action (e.g. repentance) that will repair the wrong committed and bring one "into good graces" with the offended individual or group. According to MacDonald & Shaw (2004), we learn to avoid inclusion-threatening situations to limit the amount of "exclusion threats" we face in interpersonal contact. "Feelings of pain can provide a strong sense of [aversion] that, when paired with exclusion-threatening situations, can motivate avoidance of such situations" (MacDonald et al., 2004, p. 6).

Responses to Exclusion Warnings

These responses help us "regulate inclusionary status" to maintain our social image:

Because of the strong relationship between pain and threat-defense response mechanisms, pain affect should provide a pathway by which social exclusion cues could trigger quick, defensive reactions to regulate inclusionary status. (MacDonald et al., p. 204)

In a study presented at the Seventh Annual Sydney Symposium of Social Psychology in 2004, Norbert Kerr of Michigan State University called *social exclusion* "the social death penalty." With such emotionally charged and powerful language associated with social exclusion, it is easy to see why we would seek to find any method available to avoid being socially excluded (Kerr, 2004, p. 2).

Leadership Styles

Curd (2004) outlined four basic leadership styles: the analyst, the ex-

presser, the director, and the influencer, which are being correlated to the four social codes.

- Analyst (comfortable introvert): compliant, conservative, systematic, withdrawn, organized.
- Expresser (uncomfortable introvert): values communication, avoids confrontation, conforms to surroundings, has a very personalized and individualized office space.
- Director (comfortable extrovert): quick thinking, action-oriented, irritated by indecision and tends to take charge of a stressful situation.
- Influencer (uncomfortable extrovert): expressive, creative, trendy, spontaneous, seek recognition, easily irritated by structure (Curd, 2005, p. 1).

Methods

Fifty students at a western U.S. undergraduate institution were surveyed. Five introductory classes were polled at the institution, all of which have a variety of students from all the various disciplines at the institution. In addition, an equal number of surveys were dispersed in every class (10) to protect the validity and reliability of the findings, and the female:male ratio was I:l.

Results

Image Maintenance

Overall, 76 percent of the respondents indicated that personal image plays a big role in social maintenance. In the verbal responses, outward appearance and first impressions seemed to be a theme with the respondents who answered with a high image maintenance need. A response from a 19-year-old female UE respondent who answered with a 9 on the IM scale supports that notion:

Image maintenance is important because one's appearance is the first thing people notice. One's appearance portrays a lot of their personality.

A response from a male CE respondent who answered 10 on the IM scale points out that image is important on many different fronts:

Image is the first thing the people notice about you so...how you carry yourself can determine which opportunities will be available to you.

In contrast, those who answered lower on the scale more often than not listed self-esteem related traits as their reason for low image maintenance. A response from a male CI who answered with a l on the IM scale supports that notion:

If someone doesn't like you for your personality, you don't need to like them

Leadership Styles

Sixty-four percent of the respondents matched the social personality to the leadership style. Seventy-three percent (1% SD) matched social code to the leadership style.

The females polled in this section answered with more extroverted traits than their male counterparts, bringing Tannen's "Genderlect" theory into question.

Discussion

The findings in the leadership styles section question Tannen's "Genderlect" because the females were found to be more extroverted than their male counterparts. Overall, two relationships were found:

- 1. Image maintenance and social needs have a positive relationship: one goes up as the other goes up.
- 2. Social code and leadership style have the same positive relationship.

Females answered higher on the social coding scale in the leadership style section to indicate they have a higher need for status in the workplace than their male counterparts. These findings bring "Genderlect" theory into question, giving reason for further research to be conducted on a larger scale to support or debunk the findings of this study.

Direction of Future Research

Research will be conducted to support or debunk the leadership styles portion of this study and look into the possibility of a "chart of relational possibilities," outlined in Table 2:

Table 2 Chart of Relational Possibilities: Intermixing the social codes in social relationships brings up ten relational possibilities for future study.

CI+CI	CI+UI	CI+UE	CI+CE
	UI+UI	UI+UE	UI+CE
		UE+UE	UE+CE
			CE+CE

References

- Alexander, R. D. (1986). Ostracism and Indirect Reciprocity: The Reproductive Significance of Humor. *Ethology and Sociobiology*, 7, 253-270. http://www.be-press.com/context/gruterclassics/article/1034/viewcontent/
- Curd, S. (2004). Manage Personalities, Not Just People. *Review of Ophthalmology*, *11*(8). Retrieved June 21, 2007, from http://www.revophth.com/index.asp?page=1_568.htm
- Devito da Cunha, A., & Greathead, D. (2007). Does Personality Matter? An Analysis of Code-Review Ability. *Communications of the ACM*, 50(5), 109-112.
- Kerr, N. L. (2004). Avoiding the Social Death Penalty: Ostracism and Cooperation in Social Dilemmas. Michigan State University.
- MacDonald, G., & Shaw, S. (2004). Adding Insult to Injury: Social Pain Theory and Response to Social Exclusion. University of Queensland, 15.
- MacDonald, G., & Leary, M. R. (2005). Why Does Social Exclusion Hurt?: The Relationship between Social and Physical Pain. *Psychological Bulletin*, 131(2), 202-223.
- Ridgeway, C. L. (1999). Social Difference Codes and Social Connections: 1999 Presidential Address to the Pacific Sociological Association. *Sociological Perspectives*, 43(1), 1-11.

Van Baren, J., Ijsselsteijn, W., Markopoulos, P., & Eindhoven, N. R. (2007). Measuring Affective Benefits and Costs of Awareness Systems Supporting Intimate Social Networks. *Philips Research Eindhoven*. 13-21.

HEALTH PROMOTION AND HUMAN PERFORMANCE

A Psychological Analysis of College Basketball Players

James Bokinskie

Faculty Mentor: Daniel Balderson

Abstract

This research is an attempt to understand and analyze psychological characteristics among college basketball athletes during competition. A self-reporting profile (CAP) was administered among the men's (N=17) and women's (N=15) basketball teams at Weber State University. Results indicated that 50% of college basketball players considered themselves as mentally tough, and the other 50% considered themselves moderately mentally tough. None of the athletes considered themselves as either mentally weak or extremely mentally tough. Also, 44% more males indicated being mentally tough (71%) than females did (27%). The strongest characteristic, on a scale from 1 to 10, among both sexes was being competitive (9.06). The weakest characteristic among males was their ability to relax (7.35) and among females was having patience (5.4). In addition, a positive correlation (0.626) was discovered, which states that 39% of the variation in the total score on the CAP is due to the number of years having competed in basketball.

Introduction

Psychology is the study of the mind and behavior. Research in psychology is quite prevalent and attempts to understand and explain behavior, emotion, and thought. The field ranges from academic studies to applied sciences. Areas of interest among psychology include mental health treatment, self-help, ergonomics, fields relating to health science, and performance enhancement. Performance enhancement is included in the field of sport psychology. The practice of sport psychology is used at high levels of competition for helping improve athletes' performance. Sport psychology is based on the idea that performance can be improved by cognitive conditioning strategies. Several studies have proven the effectiveness of these strategies, such as Weinberg & Williams' (2001) study Applied sport psychology: Personal growth to peak performance. According to the study done by Weinberg and Williams (2001), the practice of sport psychology is an effective practice in working with the world's best athletes. James E. Loehr's (1994) The New Toughness Training for Sports, discusses some of his methods and experiences with using psychological techniques and evaluations in working with athletes over several years. Just as Weinberg & Williams (2001), and Loehr (1994) have proven, I hypothesize that the results from my study will support that there is a real, continued need for sport psychologists in order to improve weak mentality and peak athletic performance.

Methods

Thirty-two CAP profiles were administered among college basketball players at Weber State University. All of the profiles were returned and data was entered into NCSS (Number Cruncher Statistical Systems, 2007) to be evaluated. With the software, each athlete's answers were summed for a total score. There were twenty-six different adjectives included in the CAP along with their opposite term (i.e. patience vs. impatience), and the athletes indicated how strong or weak they felt in each category by rating themselves from 1 to 10, which made a maximum score of 260. Then each total score was recorded to determine if each athlete was extremely tough (250-260), mentally tough (208-250), moderately tough (156-208), or mentally weak (0-156). Also, each categorical score was analyzed in order to find the mean for each adjective. Finally, with information received on how many years each athlete had played his/her sport, Pearson's correlation method was used to determine if there was a relationship between how many years of basketball had been played in their lifespan and how mentally tough they were.

Instrument

The profile that was used came from James E. Loehr's (1994) The New Toughness Training for Sports and is called the Competitive Adjective Profile (CAP). The profile asks the athletes to rate themselves on a scale of I (indicating an extremely weak characteristic) to 10 (indicating an extremely strong characteristic) for how they view themselves during competition for twenty-six different cognitive adjectives. The profile allows a maximum possibility of 260 points and classifies athletes into categories of mental toughness (extremely tough, tough, moderately tough, and weak). In addition, data provides evidence to determine what characteristics are weak, moderate, and/or tough. Loehr (1994) uses this profile with his clients for helping them obtain the goal of being extremely mentally tough. The goal for the athlete is to be able to report no less than a nine on each characteristic. In order to improve their overall score, athletes are advised to take the survey once a month and then focus on improving the four weakest characteristics reported for that month.

Results

Athletes' Totals

The study indicated that 50% of college basketball players considered themselves mentally tough, while 50% considered themselves moderately tough, and none considered themselves mentally weak or extremely tough. The mean of total scores was 210.75, which falls into the category of mentally tough. The standard deviation (SD) was 20.25 and the range of scores was 170 - 250 = 80.

Male

Seventy-one percent of males considered themselves mentally tough, while the remaining 29% considered themselves moderately tough. The mean for total scores among males was 221.12 (mentally tough). The SD was 20.51 and the range was 170 - 250 = 80. On average, male players had competed in their sport for 13.29 years.

Female

Twenty-seven percent of females considered themselves mentally tough, and the remaining 73% considered themselves moderately tough. The mean for total scores among females was 199 (moderately tough). The SD was 12.19 and the range was 178 – 217 = 39. On average, female players had competed in their sport for 8.1 years.

Male/Female Comparison

Forty-four percent more males considered themselves mentally tough

than females. The difference in total means for men (221.12) and women (199) favored men to be more mentally tough on an average of 22.12.

The SD indicated that scores of males (SD 20.51) varied more than scores of females (SD 12.19) on an average of 8.32 (see figure 1).

Discussion

Sport psychology practices are utilized among today's greatest performers right alongside the many other strategies used for helping athletes to reach peak performance. Although, sport psychology is often the practice most

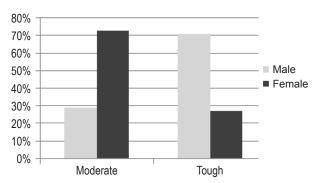


Figure 1 Compares the percentage of males from females of total scores on CAP. The categories of extremely tough and mentally weak did not receive scores, so they are not noted in the diagram.

Table 1 Illustrates the three strongest and three weakest characteristics discovered by the CAP for all athletes (N=32).

Strongest	Weakest
Competitiveness (9.16)	Patience (6.88)
Coachable (8.69)	Relaxing (6.97)
Committed (8.69)	Even Tempered (7.19)

Mean for all twenty-six categories = 8.12, Standard Deviation of 0.78

neglected (Athletic Insight, 2007). Therefore, this research further supports that there is a real, continued need for sport psychology, in order to improve weak mentality and elevate performance. Among the findings of this research, it is fair to report that none of the basket-ball players tested fell into the category of extremely mentally tough. Rather, overall, the male players considered themselves mentally tough, and the females considered themselves only moderately tough. The mean for males was 8.5 and 7.6 for females. These results report an obvious room for improvement in weak mentality. Male athletes reported low scores for the ability to remain poised and relaxed, demonstrate strong body language, remain even-tempered, and be energetic. Females reported low scores for being patient and relaxed, remaining even-tempered, being emotionally stable, and having discipline. Re-

Table 2	Illustrates the strongest and weakest characteristics for males (N=17) and females
(N=15) i	indicated from the CAP.

•	MALE	FEMALE			
Strongest	Weakest	Strongest	Weakest		
Maturity (9.24)	Relaxing (7.35)	Competitive (9.27)	Patience (5.4)		
Team Play (9.24)	Energetic (7.71)	Aggressive (8.60)	Relaxing (6.53)		
Coachable (9.18)	Strong Body Language (7.76)	Motivated (8.47)	Even Temper (6.53)		
Focused (9.18)	Even Temper (7.77)	Team Play (8.27)	Discipline (6.73)		
Competitive (9.06) none		Coachable (8.07) Emotionally Stable (7.00)			
Total	Mean 8.5	Tota	Il Mean 7.6		

sults indicated that athletes have room to improve on several of the characteristics within the CAP. Also reported, was that the longer an athlete has played his/her sport, the more likely his/her total score is to increase. Results indicate that there is a need for sport psychologists in college basketball. Methods and conditioning practices employed by sport psychologists could provide athletes with the opportunity to transform their weaknesses into strengths, creating a more competitive athlete and team. Recommendations for furthering this research would be to allow the two surveyed basketball teams to work with a sport psychologist for a period and then be re-evaluated with the CAP. This would provide concrete evidence that these practices are not only beneficial, but also vital in reaching peak performance along with reaching extreme mental toughness.

References

Athletic Insight. *The Online Journal of Sport Psychology*. (2007). Retrieved November 11, 2007, from http://www.athleticinsight.com/

Loehr, J. (1994). The New Toughness Training for Sports. New York: Penguin Group.

Loehr, J. (1994). Competitive Adjective Profile (CAP).

Weinberg, R. S., & Williams, J. M. (2001). Integrating and implementing a psychological skills training program. *Applied sport psychology: Personal growth to peak performance*, 347-377.

CLINICAL LABORATORY SCIENCES

Effects of Hemolysis and Lipemia on Immunoassay Results

Van Aston & Aaron Harper*

Faculty Mentors: William Roberts & William Zundel

Abstract

Hemolysis and lipemia are common interferents in clinical laboratory tests. In high enough quantities they can either positively or negatively interfere with testing, meaning that the analyte values being measured may not be accurate. The purpose of this study was to determine what levels of lipemia and hemolysis interfere with specific immunoassays. The assays tested include 2 categories: competitive and non-competitive (sandwich) assays. The competitive assays include T3, T4 and testosterone. The non-competitive assays include: TSH, SHBG and IgE. Each analyte was tested with multiple concentrations of interferences in duplicate. All testing was performed on the Roche Diagnostics Modular E170 analyzer at ARUP Laboratories in Salt Lake City, Utah.

Clinically significant findings are determined at a concentration of interferent that affects recovery of the analyte by +/- 10%. Analytes were measured in samples gathered from ARUP storage. The samples were pooled together and tested to establish a baseline. Each concentration was prepared from this baseline pool and compared against it. Tests that showed significant effects from hemolysis were IgE, T3 and T4. Tests that showed significant effect from lipemia were: SHBG, T3, T4 and testosterone. Some reagent manufacturers do not provide information on common interferences with immunoassays. The result of this study encourages individual laboratories to determine acceptable ranges for lipemia and hemolysis interferences for their immunoassays.

Introduction

Three common types of interferences are present in serum or plasma samples tested in the clinical laboratory. They are hemolysis, icterus, and lipemia and they can be quantified by measuring a serum index value (SI).

Lipemia is primarily due to high concentrations of triglycerides

^{*} Eccles Undergraduate Research Travel Award

present in chylomicrons in the patient's blood. This may cause the serum to take on a white or milky appearance. Lipemia is known to effect photometric assays, when the concentration of lipids in the blood increases the amount of light scattered when passing through the sample also increases. But the study of lipemic interference with immunoassays has been limited. One purpose of this study was to determine if lipemia has an effect on the antibody-antigen reaction that takes place in an immunoassay. This interference is expected to be worse with competitive assays than with non-competitive assays, which will be discussed later. Fortunately, the lipemia problem is easily solved. By subjecting the sample to ultracentrifugation, the sample can be spun down at a rate that forces the lipids to the surface and a clear sample can be retrieved.

Hemolysis is due to the hemoglobin that is released from a red blood cell after the cell is broken, commonly referred to as cell lysis. If the sample is hemolyzed, this is evident by a red color appearing in the serum. The cell may lyse during the initial draw due to poor phlebotomy technique, or after the draw because the sample was stored at an inappropriate temperature (frozen). Hemolysis has also been tested extensively in photometric assays, but only limited testing has been performed on immunoassays. Hemolysis is more difficult to deal with in a sample; there is no treatment to reduce hemolysis like there is for lipemia. A new sample may be requested or a footnote may be placed with the sample result informing the physician that the results may be affected by excessive hemolysis.

Methods

All testing was performed on the Roche Diagnostics Modular E-170. Roche designed these machines to use a ruthenium complex as their electrochemilluminescence marker. This marker is used in all assays tested. The assays tested are divided into 2 types: competitive immunoassays and non-competitive immunoassays. These testing methods are significant because most hormone testing performed in a clinical or hospital setting uses this technology. The competitive assays that were tested include triiodothyronine (T3), thyroxine (T4), and testosterone. The non-competitive assays include thyrotropin (thyroid

stimulating hormone, TSH), sex hormone binding globulin (SHBG), and immunoglobulin E (IgE).

Competitive assays

A competitive assay uses a solid phase particle, with antibody to the analyte being tested bound to its surface. The reagent contains a chemically labeled ligand that competes with the analyte. The machine detects the amount of light given off by the marker and calculates the amount of analyte. The concentration of analyte in a sample will be inversely proportional to the amount of light given off. In other words, if more chemically bound ligand attaches to the solid phase, there is less analyte available in the sample. Inversely, if less ligand is bound, then less light is given off, meaning there is a greater amount of analyte in the sample.

Non-competitive assays

The non-competitive assay, also called a sandwich assay, uses a solid phase particle similar to the competitive assay. The particle has antibody to the analyte bound to its surface. When the solid phase comes into contact with the sample, it binds to the analyte. The solid phase is then sent through a wash cycle that removes excess sample. Chemically marked antibody to the analyte is then added which binds to another part of the analyte that is already attached to the surface of the solid phase particle. The machine then reads the amount of light given off. This signal is proportional to the amount of analyte in the sample. If the amount of light given off is high then the concentration of analyte is high.

Two sets of samples were prepared from the same sample pool. Natural hemolysate was obtained by freezing whole blood samples then spinning down the remaining cellular materials. The hemolysate was placed in a Beckman-Coulter hematology analyzer. The measured hemoglobin was 26,000 mg/dL. 2%, 4%, 8% and 16% dilutions were made using the prepared hemolysate. Testing was performed in duplicate on each dilution and the results from each test were averaged. Testing was also performed on the serum pool to provide a baseline. Two more sets of samples were prepared using Intralipid. Intralipid is

used to simulate lipemia in the serum. Ratios of 1:5, 1:10, 1:20 and 1:40 were prepared. Testing was performed in duplicate and the results were averaged. Each analyte value was plotted against the lipemia or hemolysis values to determine where the clinically significant interference began.

Results

Clinically significant interference is determined at 90% recovery of the anlayte. Therefore, if 90% or more of the analyte's total concentration was detected, then the lipemia or hemolysis concentrations had no effect. If the recovered analyte's percentage is less than 90%, then the lipemia or hemolysis concentration had a clinically significant effect on the assay. The following values represent the concentrations for each analyte where the 90% interference was reached. Interference with IgE occurred at 4,830 mg/dL Hb and 2,776 mg/dL lipemia. SHBG showed significant interference at 6,616 mg/dL Hb and 1,778 mg/dL lipemia. Significant interference for IgE occurred at 4,632 mg/dL Hb and 2,066 mg/dL lipemia. T3 interference occurred at 10,348 mg/dL Hb and 1,135 mg/dL lipemia. Interference for T4 occurred at 6,312 mg/dL Hb and 1,763 mg/dL lipemia. Significant interference for T8H showed at 10,000 mg/dL Hb and 695 mg/dL lipemia. (Please refer to tables in the Results section.)

With the results of this data, acceptable criteria for samples can be established. Additional testing will need to be done to establish a specific interference for other analytes on the E170 analyzer.

Discussion

All of the testing was performed on one analyzer at one laboratory. These results are valid for the analyzer and assays that we evaluated. Other laboratories are encouraged to perform their own interference studies to determine what levels of hemolysis and lipemia affect their testing.

Outside the research laboratory where patient samples have many different appearances, the extreme amounts of both hemolysis and lipemia are often found. Patients can exhibit lipemia values in excess of 3,000 mg/dL. The tests that showed significant interference will

need to be ultracentrifuged to obtain an accurate analyte value. Gross hemolysis presents a significant problem since nothing can be done to change the hemoglobin levels in a sample. Often there is no other sample available for testing. The physician will need to be notified of the potentially inaccurate values reported by the lab due to excessive hemolysis. Laboratories using this type of testing need to examine the limits of interference on their tests. Most manufacturers do not do extensive testing in this area.

It is interesting to note the effects of hemolysis on Testosterone and T3. With each of the analytes, an 8-10% change is expected with the corresponding dilution factors. Testosterone and T3 never fell below the 90% expected range, even with a 16% dilution. This suggests that hemolysis is actually showing a positive interference on these analytes; the free hemoglobin is making up for the decrease in analyte due to the dilution factor.

Intralipid was used to substitute for lipemia in each sample. Intralipid is a close approximation of natural lipids, but is not exact. When possible, actual native triglycerides may provide more accurate results [1]. Due to the difficulty of obtaining large amounts of native triglycerides, this study used Intralipid exclusively.

Normal saline was supplemented with both hemolysis and lipemia as controls to ensure that none of the analytes tested were present in the interferences themselves. Each control showed negative values for all analytes.

Table 1 Effects of Hemolysis.

Analyte	Hemoglobin Concentration	Direction of Interference
IgE	4,830 mg/dL	Negative
SHBG	6,613 mg/dL	Negative
TSH	4,632 mg/dL	Negative
Т3	10,348 mg/dL	Positive*
T4	6,312 mg/dL	Negative
Testosterone	10,000 mg/dL	Positive*

^{*} Represents clinically significant interference.

Table 2 Effects of Lipemia.

Analyte	Lipemia Concentration	Direction of Interference
IgE	2,776 mg/dL	Negative
SHBG	1,778 mg/dL*	Negative
TSH	2,066 mg/dL	Negative
T3	1,135 mg/dL*	Negative
T4	1,763 mg/dL*	Negative
Testosterone	695 mg/dL*	Negative

^{*} Represents values commonly found in patient samples in hospital settings.

Testing for this study involved heavily lipemic and hemolytic samples. Testosterone showed significant interference from both lipemia and hemolysis. T3 showed significant interference from hemolysis. Reagent manufacturers perform only limited testing for these immunoassays. This study should encourage every lab that uses this methodology to establish their acceptable limits for hemolysis and lipemia.

Acknowledgments

The ARUP Institute for Clinical and Experimental Pathology provided complete support for this study. William Owen, an MT at ARUP Research and Development, provided assistance during the testing process. Dr. William Roberts from the Department of Pathology is responsible for initiating and overseeing the research process. William Zundel provided important insight as the Faculty Mentor for this project.

References

Bornhorst, J. A., Roberts, R. F., & Roberts, W. L. (2004). Assay-specific differences in lipemic interference in native and intralipid-supplemented samples. *Clinical Chemistry*, 50, 2197-2201.

Biddle, D. A., Dasgupta, A., & Wells, A. (2001). Negative interference of bilirubin and hemoglobin in the MEIA troponin I assay but not in the MEIA CK-MB assay. *Journal of Clinical Laboratory Analysis*, 15(2), 76-80.

Clark, T., Ma, Z., & Scott, M. (1997). Effects of Hemoglobin and Perflubron-

- based Oxygen carriers on common clinical laboratory tests, *Clinical Chemistry*, 43(9), 1732-1737.
- King, M., Rogers, M., & Snyder, J. (2004). The impact of hemolysis on Ortho-Clinical Diagnostic's ECi and Roche's elecsys immunoassay systems, *Clinica Chimica Acta*, 348, 181-187.
- Kwon, H., Min, K., & Seo, E. (2003). The influence of Hemolysis, Turbidity and Icterus on the measurements of CK-MB, Troponin I and Myoglobin, *Clinical Chemistry Lab Med*, 41(3), 360-364.

CLINICAL LABORATORY SCIENCES

The Human Metapneumovirus in Pediatric Patients of Utah

Clinton Potter & Devin Christensen*

Faculty Mentor: Scott Wright

Abstract

Nasopharyngeal aspirates were obtained from 120 patients, newborn through five years of age, which were hospitalized with suspected respiratory infections. Hospitals routinely test these types of patients using a respiratory panel consisting of Respiratory Syncitial Virus (RSV), Influenza A & B, Adenovirus, and Parainfluenza 1, 2, & 3. Very recently, hospitals began testing for the human Metapneumovirus (hMPV) using Direct Florescence Antibody (DFA) testing. The objective of this research was to confirm the DFA results for the presence or absence of hMPV in the nasal aspirates using a newly published methodology which involved 1) extracting the RNA, 2) creating cDNA using Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR), and then 3) Polymerase Chain Reaction (PCR) amplification of the sample using primers specific for hMPV (Gray et al., 2006, p. 347). After completion of the RT-PCR testing on each sample, the DFA results, which were provided by a local hospital, were compared to the RT-PCR results. This research study confirmed a 98.3% correlation between DFA hMPV results and hPMV results by PCR.

Introduction

In the pediatric population, Respiratory Syncitial Virus (RSV), parainfluenza viruses, and influenza viruses account for a majority of the cases of respiratory viral infections that lead to bronchitis and pneumonia (Boivin, Abed, & Pelletier, 2002, p.1330) (Van den Hoogen, 2001, p. 720). However, in about 15% to 34% of respiratory cases the cause of the illness remains unknown (Esper, Boucher, & Weibel, 2003 p. 1407). This leads to the conclusion that another causative agent is involved.

A new paramyxovirus associated with respiratory tract infections was discovered in the Netherlands in 2001, Human Metapneumovirus

(hMPV) (Van den Hoogen, 2001, p. 719) (Viazov, Ratjen, Scheidhauer, & Fiedler, 2003, p. 3043). Recent studies have suggested that this virus has been in circulation for over 50 years, but it has not been detected due to limitations in viral testing (Van den Hoogen, 2001, p. 719) (Williams, 2005, p. 570). A number of recent developments have enabled clinical laboratories to begin testing patient samples for hMPV. Direct Fluorescent Antibody (DFA) testing was introduced in recent years, and following the characterization of the viral genome, an alternative method to DFA was published which then allowed for testing by PCR (Barry-Murphy, Setterquist, & Gray, 2006, p. 2). Through the use of these two methodologies, it has been proven that 5-10% of patients with respiratory symptoms are infected with hMPV (Boivin, Abed, & Pelletier, 2002, p. 1331). The objective of this research project was to validate this new method of PCR by using a series of patient specimens that have been confirmed in a hospital laboratory as either positive or negative using DFA testing.

Methods

This research was conducted at Weber State University in the department of Clinical Laboratory Sciences. Nasopharyngeal aspirates of 120 patients and corresponding DFA panel results were donated from a local hospital. The nasal samples were processed using RNA extraction, reverse transcriptase, and PCR amplification methods outlined below.

RNA Extraction

Reagent preparation and RNA extraction procedures were performed using QIAamp Viral RNA Mini columns (Qiagen, 2005, p. 15-25). 560 μL prepared Buffer AVL was mixed with 140 μL nasal washings. The solution was incubated at room temperature for 10 minutes and centrifuged for 30 seconds. 560 μL ethanol was added and mixed by pulse-vortexing for 15 seconds. RNA was separated from the extract solution using the QIAamp Mini spin column. Following centrifugation, 500 μL Buffer AW1 and AW2 were added and centrifuged. The DNA was eluded from the column with 40 μL buffer AVE followed by a centrifugation, and then again with another 40 μL buffer and a second centrifugation.

^{*} Phyllis Crosby Gardner Undergraduate Research Scholarship Travel Award

Reverse Transcription

Once the samples were extracted, it was first necessary to convert the RNA to DNA, which could then be used in the PCR amplification—a process known as reverse transcription. 10 μ L purified RNA, 2 μ L primer dT23VN5, 4 μ L dNTP mix, and 2 μ L water were mixed in a sterile RNase free microfuge tube, heated for five minutes at 70°C and centrifuged for 30 seconds (New England Biolabs, 2007, p. 8). After which, 2 μ L 10X RT Buffer, 1 μ L RNase inhibitor, and 1 μ L M-MuLV reverse transcriptase were added to the tube. The solutions were then incubated for 60 minutes at 42°C and then five minutes at 90°C. Lastly, 32 μ L water was added to bring the final volume to 50 μ L (New England Biolabs, 2007, p. 9).

PCR amplification

The target of the PCR amplification was a gene in hMPV referred to as F2. PCR primers were used that amplified the F2 gene. PCR was carried out using the following conditions: $5~\mu L$ cDNA, was added to $25~\mu L$ Taq 2X Master Mix, $2~\mu L$ forward F2 primer (5' - GAGCAAATT-GAAAATCCCAGACA-3'), $2~\mu L$ reverse F2 primer (5' - GAAAACT-GCCGCACAACATTTAG-3') (Barry-Murphy, Setterquist, & Gray, 2006, p. 2), and $16~\mu L$ water, for a final volume of $50~\mu L$. PCR amplification conditions were 2~m inutes at 95~C, 35~c cycles of 30~s seconds at 94~C, 30~s seconds at 52~C, and 1~m inute at 68~C. A final extension for 10~m inutes at 68~C followed the 35~c cycles (Gray et al., 2006, p. 347). The PCR products were electrophoresed on a 1%~a agarose gel and digitally photographed using 1%~e ethiclium bromide and a UV transilluminator.

Results

In Figure 1, the three stages of the analysis can be seen. The reverse transcriptase product is electrophoresed in lanes 8-12 to confirm that the transcriptase step did work. The PCR amplification of the hMPV F gene resulted in the expected 347 bp band (Gray et al., 2006, p. 347) which can be seen in lanes 14, 15, 16, and 18. Lane 17 is considered negative for hMPV despite the faint bands present.

Figure 2 is an example of a PCR amplification of 12 samples visible on the electrophoresis gel. This figure depicts both positive and

negative lanes for hMPV F gene results, corresponding to respective patient samples.

The following pie chart summarizes the results of this study. As illustrated below, DFA results obtained from the hospital were compared with PCR results on 120 patient samples analyzed in this study.

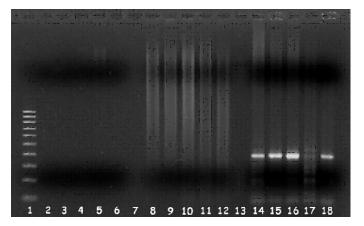


Figure 1 A 100 bp DNA marker was placed in lane 1. Lanes 2-6 contained hMPV viral RNA. Lanes 8-12 contained non-amplified cDNA from the viral hMPV RNA. Lanes 14-18 contained the PCR amplified hMPV F gene which was 347 bp. Lanes 14, 15, 16, and 18 were positive for hMPV and lane 17 was negative for hMPV.

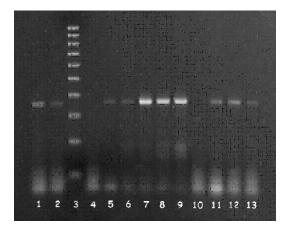


Figure 2 A 100 bp DNA marker was placed in lane 3. Lanes 4 and 10 were negative for hMPV. All the other lanes were positive for hMPV.

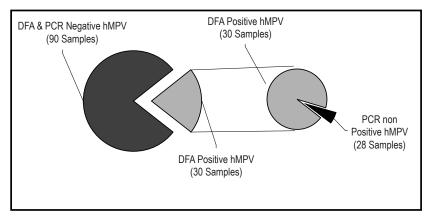


Figure 3 A total of 120 patient samples were PCR amplified. Of the 120 patient samples tested by the hospital laboratory, 90 patient samples were found to be negative for hMPV by DFA methods. These same 90 samples were found to be negative for hMPV by PCR. Of the 30 patient samples found to be positive for hMPV by DFA, 28 were found to be positive for hMPV by PCR.

Discussion

The original design of this study was to ascertain the prevalence of hMPV in pediatric patients infected with respiratory illnesses. During the course of this study, DFA testing for the presence of hMPV was implemented in local hospitals thereby changing the direction of this study to validating a newly published PCR method for hMPV and correlating results between the two methodologies.

Of the 120 samples obtained, 118 PCR results paralleled DFA testing performed by the hospital. Based on the results obtained, there is a strong correlation between PCR and DFA methods of 98.3% for the presence of hMPV in nasopharyngeal aspirates of respiratory infected patients.

It should be noted that a complete and accurate validation procedure was not performed during the course of this research due to limited funds available. In order to complete an accurate validation procedure, the results needed to be replicated multiple times. However, as previously stated, this preferred method of validation was not feasible and subsequently not performed.

Acknowledgments

This research was funded through a Phyllis Crobsy Gardner Undergraduate Research Scholarship Travel Award provided by the Office of Undergraduate Research at Weber State University. Nasal washings and hMPV DFA results were provided by an anonymous local hospital.

References

- Barry-Murphy, K., Setterquist, S., Gray, G., et al. (2006). Development of a Novel Immunoassay for the Detection of Human Metanpheumovirus in Clinical Specimens. Biotrin International, 1-2.
- Boivin, G., Abed, Y., Pelletier, G., et al. (2002). Virological Features and Clinical Manifestations Associated with Human Metapneumovirus: A New Paramyxovirus Responsible for Acute Respiratory-Tract Infections in All Age Groups. *The Journal of Infectious Diseases*, 186, 1330-1334.
- Esper, F., Boucher, D., Weibel, C., et al. (2003). Human Metapneumovirus Infection in the United States: Clinical Manifestations Associated With a Newly Emerging Respiratory Infection in Children. *Pediatrics*, *111*, 1407-1410.
- Gray, G., Capuano, A., Setterquist, S., et al. (2006). Human Metapneumovirus, Peru. *Emerging Infectious Diseases*, 12(2), 347-350.
- Peret, T., Li, Y., Couillard, M., et al. (2002). Characterization of Human Metapneumoviruses Isolated from Patients in North America. *The Journal of Infectious Diseases*, 185, 1660-1663.
- Protoscript II RT-PCR Kit Instruction Manual. (2007). New England Biolabs Inc. 8-11.
- QIAamp Viral RNA Mini Handbook 2nd ed. (2005). Qiagen. 15-25.
- Van den Hoogen, B. G. (2001). A newly discovered human pneumovirus isolated from young children with respiratory tract disease. *Nature Medicine*, 7, 719-724.
- Viazov, S., Ratjen, F., Scheidhauer, R., Fiedler, M., et al. (2003). High Prevalence of Human Metapneumovirus Infection in Young Children and Genetic Heterogeneity of the Viral Isolates. *Journal of Clinical Microbiology*, 41, 3043-3045.
- Williams, J. V. (2005). The Clinical Presentation and Outcomes of Children Infected with Newly Identified Respiratory Tract Viruses. *Infect Dis Clin N Am*, 19, 564-584.

CLINICAL LABORATORY SCIENCES

Comparison of FDA-Approved Antibiotics to Non-Prescription Mexican Antibiotics

Trina Riley, Leah Daily & Tyler Roe*

Faculty Mentor: Scott Wright

Abstract

It has become common practice for Americans to cross the border into Mexico to obtain prescription medications without a prescription. This practice is potentially dangerous because the drugs being purchased across the border are not regulated by the FDA. There is no guarantee that what the consumer is purchasing is what they think it is. The aim of this research was to compare the inhibitory action of FDA-regulated antibiotics to their Mexican counterparts. Two commonly prescribed antimicrobials, amoxicillin and ciprofloxacin, were obtained from eleven different Mexican sources. The antibiotics were tested against two common pathogens, S. aureus and E. coli. The minimum inhibitory concentration (MIC) method was used to compare the potency of the Mexican antibiotics to FDA regulated drugs from the U.S. Are the antimicrobial effects of the drugs in vitro essentially the same compared to U.S. drugs? Or are the Mexican antibiotics more effective or less effective at inhibiting bacterial growth? Results of the experiment showed no significant difference between FDA and Mexican amoxicillin. No valid conclusion could be made regarding ciprofloxacin due to testing errors.

Introduction

Americans crossing the border to purchase prescription drugs in Mexico without a prescription has become an increasing trend. A pilot study conducted by an El Paso clinic showed that 80% of the patients surveyed had crossed the border to Juarez to buy prescription drugs, stating they did so because the drugs were less expensive and no prescription was needed (Casner, 1998). This practice is dangerous because the drugs being purchased across the border are not FDA regulated. There is no guarantee that what the consumer is purchasing is what they think it is. The College of Pharmacy at the University of Arizona studied the concentration of active ingredients from drugs obtained from the U.S. and Mexico and found that the Mexican drugs were significantly less potent than their American counterparts (Karlage, 2005). According to Dr. Marv Shepherd of the University of Texas, one in five drugs obtained in Mexico is counterfeit or substandard (Doheny, 2004).

Through use of the internet, people in the U.S. no longer need to travel across the border to purchase prescription drugs without a prescription. Drugs from Mexico can be bought and mailed right to their door. Simply entering "prescription drugs for sale" into any internet search engine can bring up millions of hits for online pharmacies, many of them located outside of the United States (Henney, 1999). People can purchase anything from antidepressants to antibiotics, no questions asked. This practice is risky because there is no requirement for a physician's consultation, so many of the drugs are being taken unsupervised (Shapiro, 2005). The reason the FDA regulates these drugs by prescription in the first place is because they are dangerous to take without a physician's supervision. Self-medicating yields itself to overdosing or underdosing, and in some cases can prove lethal.

Methods

Prior to performing Minimum Inhibitory Concentration (MIC) assays, bacteria were tested for β -lactamase activity to ensure the strains were not resistant to amoxicillin or ciprofloxacin. Staphylococcus aureus (ATCC# 25923) was tested for β-lactamase activity using Hardy β-lactamase tests following the procedure outlined in the package insert. Escherichia coli (ATCC# 25922) was tested for β-lactamase activity using third generation cephalosporin disks (ceftazidime, cefoperazone, and cefpodxime) on a Kirby-Bauer plate, as recommended by Koneman et al. (1997). Both strains were determined negative for the β -lactamase enzyme.

Amoxicillin and ciprofloxacin were both purchased from three FDA-approved U.S. pharmacies, and from 14 Mexican sources. A list of these drugs is found in Tables 1 and 2. Pills were tested in duplicate from each package of antibiotics obtained. For pills in separate pack-

^{*} Weber State University Undergraduate Research Scholarship Award

 Table 1
 FDA-approved prescription antibiotics.

Amoxicillin							
Drug (Manufacturer)	mg	Exp. Date	Lot#	Pharmacy Purchased			
Generic Amoxicillin (TEVA), 3 capsules	500	7/2008	415733A	Walgreens			
Generic Amoxicillin (Sandoz), 4 capsules	500	11/2010	143488	Walmart			
Generic Amoxicillin (Sandoz), 4 capsules	250	4/2009	140932	Target			
Ciprofloxacin							
Drug (Manufacturer)	mg	Exp. Date	Lot#	Pharmacy Purchased			
Generic Cipro (Ranbaxy), 4 tablets	500	9/2008	1696836	Walmart			
Generic Cipro (Ranbaxy), 4 tablets Generic Cipro (Ranbaxy), 4 tablets	500	9/2008	1696836 1705228	Walmart Target			

 Table 2
 Non-prescription Mexican antibiotics.

Amoxicillin				
Drug (Manufacturer)	mg	Exp. Date	Lot#	Pharmacy Purchased
Amoxicillin A Omacid (Laboratorios Kenedot) 100 capsules	250	10/2008	357	Cabo San Lucas, Mexico
Vandix (Farmaceutica Wandel) 24 capsules	250	9/2008	WCI06332	Mexmeds4you.com
Vandix (Farmaceutica Wandel) 24 capsules	250	9/2008	WCI06333	Medsmex.com
Amoxicillina (Merck Genericos) 24 capsules	500	3/2008	H03104	Medsmex.com
Pamoxab (Genetica Laboratorios) 100 capsules	500	11/8/2008	61171	Pey Pharma Distrubuidora Tijuana, Mexico
Penticlox (Productos Maver) 12 capsules	500	1/11/2008	B0626	Garcirama av. Padilla anda- dor, Tijuana, Mexico
Lumox (IQFA*) 12 capsules	500	6/2007	1310405	Garcirama via dela juventud ote, Tijuana, Mexico
Amoxil (GlaxoSmithKline Mexico) 12 capsules	500	10/2009	0O3BG032	The Medicine Company Tijuana, Mexico
Gimalxina (Productos Farmaceuticos Collins) 12 capsules	500	7/12/2008	0240B6G	The Medicine Company Tijuana, Mexico
Vizimox (Victory Enterprises) 100 capsules	500	1/2009	AM06ABG06	Farmacia Onyx Tijuana, Mexico
Amoxifur (IVAX Pharmaceuticals Mexico) 24 capsules	500	11/2007 7/2007	511655B 507945B	Mexmeds4you.com
Amoxifur (IVAX Pharmaceuticals Mexico) 24 capsules	500	11/2007	511655B	Mymexicandrugstore.com
Amoxil (GlaxoSmithKline Mexico) 24 capsules	500	8/2008	003HG045	Mymexicandrugstore.com
Amoxifur (IVAX Pharmaceuticals Mexico) 24 capsules	500	7/2008	607516B	Medsmex.com

Table 2 continued on page 30

Table 2 Continued

Ciprofloxacin				
Drug (Manufacturer)	mg	Exp. Date	Lot#	Pharmacy Purchased
Novoquin (Farmaceuticos Rayere) 12 tablets	250	10/7/2007	5655	Garcirama via dela luventud ote, Tijuana, Mexico
Ciprain (Productos Maver) 10 tablets	250	8/22/2007	A05266	Garcirama via dela luventud ote, Tijuana, Mexico
Ciqfadin (IQFA*) 8 tablets	250	5/2007	1160105	Garciaama via dela juventud orte, Tijuana, Mexico
Ciprofloxacino GI (Laboratorios Best) 36 tablets	250	5/2008	605127	Mexmeds4you.com
Ciproflox (ALTIA/Laboratorios Sensonian) 12 capsules	250	9/2009	S6I138	Mymexicandrugstore.com
Ciprofloxacino GI (Laboratorios Best) 36 tablets	250	10/2008	61056	Medsmex.com
Ciproflox (ALTIA/Laboratorios Sensonian) 12 capsules	250	9/2009	S6I138	Medsmex.com
Genoflox (Genetica Laboratorios) 36 tablets	500	10/31/2007	51171	Farmacia Smart Buy Tijuana, Mexico
Ciproxina (Bayer de Mexico) 14 tablets	500	8/8/2011	L081	The Medicine Company Tijuana, Mexico
Clortory (Victory Enterprises) 28 tablets	500	5/2009	CL05SEG16	The Medicine Company Tijuana, Mexico
Kenzoflex (Productos Farmaceuticos Collins) Bottle had 13 tablets, label indicated 28 tablets	500	12/29/2006	R3326F4L	Farmacia Onyx Tijuana, Mexico
Kenzoflex (Productos Farmaceuticos Collins) 12 tablets	500	3/29/2008	070F6C	Cabo San Lucas, Mexico
Bacproin (Labratorios Best) 24 tablets	500	10/2008	61075	Mexmeds4you.com
Ciprofur-F (IVAX Pharmaceuticals) 24 tablets	500	8/2008	608492B	Medsmex.com

^{*}IQFA = Industrias Quimico Farmaceuticas Americanas

ages or foil cards, one pill was taken from each of the packages.

Glass tubes (13x100 mm) were labeled with the brand and generic names of the drug, the mg of the pill, and the place where it was purchased. Each pill was suspended in 10 mL of distilled water and vortexed to promote disassociation of the pill. Pills that were encapsulated or did not readily dissolve were placed in an air incubator for 10 to 60 minutes until dissolved. Once the drug was suspended, the tubes were centrifuged for five minutes at 3,270 rpm.

Two 10 mL and six 5 mL Tryptic Soy Broth (TSB) tubes were la-

beled with the same information as before, as well as the concentration of antibiotic contained in the tube after dilution (as outlined in Tables 3 and 4).

Table 3 TSB tube set up for amoxicillin.

Amoxicillin									
Tube #	1	2	3	4	5	6	7	8	
TSB Volume (ml)	10	10	5	5	5	5	5	5	
Antibiotic concentration (µg/ml)	128	8	4	2	1	0.500	0.250	0.125	

Table 4 TSB tube set up for ciprofloxacin.

Ciprofloxacin								
Tube #	1	2	3	4	5	6	7	8
TSB Volume (ml)	10	10	5	5	5	5	5	5
Antibiotic concentration (µg/ml)	64	0.1250	0.06250	0.03125	0.01563	0.007813	0.003906	0.001953

Before adding an amount (X) of antibiotic suspension to TSB tube 1 and an amount (Y) of TSB from tube 1 to tube 2, X and Y amount of TSB were removed and discarded from tubes 1 and 2, respectively. Values for X and Y are illustrated in Tables 5 and 6.

Table 5 Antibiotic dilution for amoxicillin.

	Amoxicillin	
	Χ Tube 1 (128 μg/ml)	Υ Tube 2 (8 μg/ml)
250 mg pill	51.2 μL	625 µL
500 mg pill	25.6 µL	625 µL

Table 6 Antibiotic dilution for ciprofloxacin.

	Ciprofloxacin	
	Χ Tube 1 (64 μg/ml)	Υ Tube 2 (0.125 μg/ml)
250 mg pill	25.6 μL	19.5 µL
500 mg pill	12.8 µL	19.5 µL

Next, a sample (X) of the antibiotic suspension was drawn from the supernatant of the dissolved pill into a pipette tip and inoculated into TSB tube #1, then vortexed for two to five seconds. An amount (Y) of TSB was removed from tube #1 and transferred to tube #2, and tube #2 was vortexed as before. Serial 1:2 dilutions were performed through the six remaining tubes. Tube #1 was discarded when dilutions were complete.

After performing antibiotic dilutions, a stock bacterial suspension was made from isolated colonies growing on a Sheep Blood Agar (SBA) plate. Using a sterile loop, colonies were added to a 5 mL TSB to a concentration of 3.0 x 10^8 expected CFU/mL (1.0 MacFarland) on the basis of optical density using a nephlometer. 16.7 μ L of the stock bacterial suspension (1.0 x 10^6 expected CFU/mL) was added to all TSB tubes containing antibiotic and to one 5 ml TSB blank to serve as a control. All tubes were vortexed two to five seconds before incubation in 37° C air for 18 to 24 hours. The control tube was plated quantitatively to an SBA plate using a 1 μ L calibrated loop, and the plate incubated in 37° C CO₂ for 18 to 24 hours.

The following day, tubes were vortexed as before and observed for visible growth. The minimum inhibitory concentration is the lowest antibiotic concentration which prevents visible growth (Pearson et al., 1980). Results were recorded as growth or no growth. Tubes with questionable growth were confirmed using a nephlometer. To determine purity of the culture, the first tube with growth was plated quantitatively to an SBA plate using 10 μL calibrated loops. SBA plates were incubated as described above.

Results

All amoxicillin samples tested were within the established MIC range for S. aureus (0.25 to 1 μ g/mL) (GlaxoSmithKline, 2006). For ciprofloxacin, the assays with growth were also in the established range for E. coli (0.004 to 0.015 μ g/mL) (Bayer, 2005). However, 59% (20 out of 34) of the assays had no growth in any of the tubes. Findings are illustrated in Tables 7 and 8. Shaded areas indicate tubes that contained growth. Results are shown for duplicate assays on each pill (Rep #).

Growth

No Growth

 Table 7
 Growth of S. aureas in amoxicillin.

Dag #	Antibiotic Concentration (μg/ml)							
Rep#	0.125	0.25	0.5	1	2	4	8	
1	Amoxicillin (TEVA)*						
2								
1	Amoxicillin (Sandoz)*						
2								
1	Amoxicillin (Sandoz)*						
2								
1	Pamoxab (Tij	uana)						
2								
1	Penticlox (Tij	uana)						
2								
1	Lumox (Tijua	na)						
2								
1	Amoxil (Tijua	ına)						
2							-	
1	Gimalxina (Ti	ijuana)						
2								
1	Vizimox (Tiju	ana)						
2								
1	Amoxicillin A	Omacid (Ca	abo)					
2								
1	Amoxifur (me	exmeds4you	.com)					
2			,					
1	Vandix (mexr	neds4vou.co	om)					
2	(,	,					
1	Amoxifur (my	/mexicandru	igstore.com)					
2		, varial u						
1	Amoxil (mym	exicandrug	store com					
2	Allioxii (iliyiii	calcallulugs	nore.com)					
	Amoxifur (me	ademov com)					
2	Amositui (iiit	Jusinica.colli	1					
1	Vandix (meds	amov com)						
2	variuix (meds	sillex.com)						
1	A 1 111							
	Amoxicilina (medsmex.co	om)					
2								

^{*} Indicates FDA-approved antibiotics (all others are Mexican antibiotics)

 Table 8
 Growth of E. coli in ciprofloxacin.

Growth

No Growth

Table 8	Growth of E	E. coli in ci	iprofloxac	in.				
Rep#		Antibiotic Concentration (μg/ml)						
тер #	0.00195	0.00391	0.00781	0.0156	0.0313	0.0625	0.125	
1	Cipro (Ranba	axy) - Prof.	Plaza*					
2								
1	Cipro (Ranba	axy) - Walm	nart*					
2								
1	Cipro (Ranba	axy) - Targe	et*					
2								
1	Novoquin (Ti	juana)						
2								
1	Ciprain (Tijua	ana)						
2								
1	Ciqfadin (Tiju	uana)						
2								
1	Genoflox (Tij	uana)						
2								
1	Ciproxina (Ti	juana)						
2								
1	Clorotory (Ti	juana)						
2								
1	Kenzoflex (T	ijuana)						
2								
1	Kenzoflex (C	abo)						
2								
1	Ciprofloxacin	no GI (mex	meds4you.c	om				
2								
1	Bacproin (me	exmeds4yo	ou.com)					
2								
1	Ciproflox (m	ymexicand	rugstore.co	m)				
2								
1	Ciprofloxacin	no GI (med	smex.com)					
2								
1	Ciprofur-F (n	nedsmex.c	om)					
2								
1	Ciproflox (mo	edsmex.co	m)					
2								

^{*} Indicates FDA-approved antibiotics (all others are Mexican antibiotics)

The average price per pill was calculated based on the amount paid (in dollars) and the number of pills per package. The results are depicted in Table 9.

Table 9 Average price per pill.

	Amo	ticillin	Ciprofloxacin		
	250 mg	500 mg	250 mg	500 mg	
FDA	\$0.75	\$1.92	\$1.82	\$1.36	
Mexico	\$0.18	\$0.51	\$1.33	\$1.07	

Discussion

Susceptibilities using the minimum inhibitory concentration (MIC) for amoxicillin revealed no significant difference between the effectiveness of FDA-approved antibiotics and Mexican antibiotics. Our findings contradict previous studies performed by the University of Texas and the University of Arizona, which suggest that drugs purchased in Mexico are less potent and substandard (Karlage, 2005, Doheny, 2004). One possible explanation for this contradiction may be the small sample size tested in this study. Also, prescription drugs that are more costly may have a higher likelihood of being counterfeit than the inexpensive antibiotics examined here.

Susceptibilities using ciprofloxacin revealed more variation in results. This discrepancy was present in both the Mexican and FDA antibiotics. Variation occurred on batched runs, but not on experiments with fewer than five assays. It may be possible that ciprofloxacin has a time-release action and that over time more antibiotic dissolves into solution, raising the concentration of antibiotic in the tube. Also, contamination from several bacterial species occurred on two separate occasions. One possible explanation of this is large volume testing exposed the materials to contaminants for longer periods of time. Due to the variation that occurred, no valid conclusion concerning ciprofloxacin can be made based on the data obtained.

Price differences between the FDA and Mexican antibiotics are noteworthy. On average amoxicillin costs 74.2% more in the United States. Ciprofloxacin on average is 24.5% more expensive in the Unit-

ed States. Our findings support Casner's suggestion that Americans purchasing medication in Mexico are saving money (1998).

However, safety and peace of mind may be worth the extra change one would spend for the medications in the U.S. Out of the eleven Mexican pharmacies we purchased from, only one provided any information about how to take the antibiotic. The woman at the *farmacia* pointed out that the 14 tablets in the package we were purchasing would not be a full course of the ciprofloxacin. No other instruction was given in any of the *farmacias* regarding how many pills to take and how often, what kind of infection the medication was used for, or any side affects that might be experienced. In fact, another *farmacia* knowingly sold us expired ciprofloxacin after blowing dust from the top of the half empty bottle. In contrast, each pharmacy we visited in the U.S. provided a consumer information sheet (at least one page in length) containing such information and a pharmacist who asked if there were any questions regarding the medication, or any known drug allergies.

The results found in the MIC for amoxicillin found no significant difference between FDA and Mexican antibiotic effectiveness. Results for ciprofloxacin had too much variation to make an accurate determination of effectiveness. Additional method evaluation and testing is needed to accurately test ciprofloxacin using the MIC method. Further studies using chemical analysis such as mass spectroscopy and gas chromatography may be useful in determining actual concentrations and chemical content of the antibiotics.

Acknowledgements

We would like to thank the Weber State University Office of Undergraduate Research for providing funding for this project. We would also like to thank Dr. Shawn D. McQuilken for providing research prescriptions, and Scott Wright for his input and support for this project. Thanks to Kent Criddle for his knowledge and expertise in ordering supplies.

References

- Bayer Pharmaceuticals Corporation. (2005). *Cipro prescribing information*. Retrieved January 19, 2007, from http://www.univgraph.com/bayer/inserts/ciprotab.pdf.
- Casner, P., & Guerra, L. (1998). Purchasing prescription medication in Mexico without a prescription: the experience at the border. *The Western Journal of Medicine*, 156(5), 512-16.
- Doheny, K. (2004). Think twice before buying prescription drugs in Mexico. Los Angeles times. Retrieved September 12, 2006, from http://www.latimes.com/features/health/medicine/la-tr-healthy8aug08,1,2053803.column?coll=la-health-medicine.
- GlaxoSmithKline United States. (2006). *Amoxil prescribing information*. Retrieved January 19, 2007, from http://us.gsk.coom/products/assets/us_amoxil.pdf.
- Henney, J., Shuren, J., Nightingale, S., & McGinnis, T. (1999). Internet purchase of prescription drugs: buyer beware. *Annals of internal medicine*, 131 (11), 861-62.
- Karlage, K. L., & Myrdal, P. B. (2005). Comparison of three pharmaceutical products obtained form Mexico and the United States: a case study. *Drug Development and Industrial Pharmacy*, 31(10), 993-1000.
- Koneman, E. W., Allen, S.D., Janda, W. M., Schreckenberger, P. C., & Winn, W. C. (1997). *Color atlas and textbook of diagnostic microbiology* (5th ed.). Philadelphia, PA: Lippincott.
- Mahon, C. R., & Manuselis, G. (2000). *Textbook of diagnostic microbiology*, (2nd ed.). Philadelphia, PA: Saunders.
- Pearson, R. D., Steigbigel, R. T., Davis, H. T., & Chapman, S. W. (1980). Method for reliable determination of minimal lethal antibiotic concentrations. Antimicrobial Agents and Chemotherapy, 18(5), 699-708.
- Shapiro, A. (2005). DEA cracks down on illegal web sales of narcotics. NPR morning edition. Retrieved September 12, 2006, from http://www.npr.org/templates/story/story.php?storyId=4715097.

CLINICAL LABORATORY SCIENCES

The Effects of Stress on Platelet Function

Alisha Weiss, Nathan Fenn & Majak John*

Faculty Mentor: Kara Hansen-Suchy

Abstract

The purpose of this study is to determine if mental stress has an effect on platelet function. The hypothesis is that mental stress increases platelet function in the body, thereby contributing to clot formation in blood vessels. We correlated the effects of acute mental stress to platelet function. This was done by taking a baseline sample of blood from 31 healthy male and female students and performing platelet function studies on an advanced platelet function analyzer. A second sample was collected after a series of modified Trier Social Stress Tests (TSST) were administered. We found after analyzing results that the mean post stress results did drop slightly after the TSST, indicating possible increased function; however, there was not a statistically significant difference between pre-stress and post-stress test samples.

Introduction

Heart disease remains one of the leading causes of death in men and women throughout the United States (Brydon, Magid, & Steptoe, 2006, p. 113). Diet, lack of exercise and genetics have been proven factors in its cause; however, in the past few years, evidence shows that stress may be a fourth factor in increasing the risk. Recent studies have shown that stress may increase platelet activity and platelet-leukocyte interaction through changes via the sympathetic nervous system (Aurigemma et al., 2005, p. 25) (Erusalimsky, Edwards, & Brydon, 2003, p. 57) (Lederbogen, Reka, Maria, 2004, p. 55) (Gibson et al., 2006, p. 456) (Strike, Kesson, & Daisy, 2006, p. 4322). The objective of this research project was to evaluate both baseline (pre-stress) and poststress levels of platelet function using laboratory instrumentation to determine if there are measurable differences.

^{*} Weber State University Undergraduate Research Scholarship Award

The full contribution of stress on clot formation is still unknown, but it is thought to increase platelet activity through chemical involvement via the sympathetic nervous system. The active platelets then bind to white blood cells, causing them to release a variety of chemicals that cause complex changes that can lead to damage of the vessel lining, which in turn attracts migration of more platelets and white blood cells that lead to a formation of a clot (Brydon, Magid, & Steptoe, 2006, p. 113). If an increase in platelet activity results in a subsequent increased risk of clot formation, then reducing even temporary stress in our lives could perhaps lower the risk of developing heart attacks or strokes later in life.

In this study we compared the effect of temporary stress stimulation on the effect of platelet function. The hypothesis was that there would be an increase in platelet activity after a stress stimulation session as compared to baseline levels. Previous research has been inconclusive, in part due to test methodology being dependent on operator skill and other variables related to individual test subjects (Erusalimsky, Edwards, & Brydon, 2003, p. 57). With the advent of new methodology, this study tried to eliminate some of the previous researchers' variables by using a more reliable and reproducible platelet analyzer, the PFA- 100^{TM^7} , and using a more modern and standardized psychological stress test, a Modified Trier Social Stress Test (TSST) (Williams & Haggerty, 2004, p. 277).

Methods

We tested over the course of several days, a total of 31 participants composed of college-aged students (18-30 years), presumably less affected by atherosclerosis, high blood pressure, heart disease, and other disorders that may have predisposed platelet interaction. Subjects were volunteers from allied health classes. A modification of the Trier Social Stress Test (TSST) was used in order to induce stress in the subjects (Williams & Haggerty, 2004, p. 277). The modified TSST was 15 minutes in length and was composed of three tests under a mock trial of student performance. In each TSST session, approximately ten students were led to believe that these tests were an indicator of how they will perform in their desired academic program.

All students were informed that the research project was totally voluntary. To avoid interference of experimental effects, the participant was instructed not to smoke, eat, exercise, drink alcohol or low-pH soft drinks for one hour immediately before the TSST, or take aspirin for 24 hours before the TSST (Williams & Haggerty, 2004, p. 277). All participants were provided with questionnaires regarding prior medical history of self and family, use of aspirin or other medications known to affect platelet function, and their personal perception of stress. Since certain drugs, such as aspirin, affect platelet function, the volunteers were pre-screened for use and those values were removed from the study if volunteers were on those medications.

The participants completed questionnaires along with Institutional Review Board (IRB) release forms to help determine if they complied with these limitations. All TSST sessions for all groups were completed at the same time of day to ensure stability of results.

To establish a baseline, blood samples and vital signs were taken prior to the TSST. It has been determined by a prior study that maximum platelet changes will occur approximately thirty minutes after a stressful episode (Erusalimsky, Edwards, & Brydon, 2003, p. 57). Therefore, all participants' vital signs were again taken immediately after the TSST and the perceived level of stress questioned while blood was drawn at 30 minutes post session. Data has been collated and analyzed using Microsoft Excel software.

Before the TSST began, the applicants filled out a pre-stress questionnaire, and their vital signs and blood samples were taken and run on the Coulter Max- M^{TM} and in duplicate on the PFA-100 to assess pre-stress platelet function. The first test involved a five-minute timed arithmetic calculation that was composed of subtraction by 13 starting at 1,022. The participants were informed that if an error was made, they had to start again from 1,022. The participants were told that this was a measure of their ability to perform well under pressure.

Immediately following this test, the ten participants were given five minutes to complete an essay explaining their strengths, weaknesses, goals, and previous accomplishments. Participants were told that they would be graded on organization, grammar, spelling, and penmanship. For the final test, the participants were expected to pre-

pare an impromptu speech on the topic of why they are a better candidate for their desired program than the other applicants. Although the actual speech never materialized, they were told that they had five minutes to prepare before they had to speak before a panel of professors and the other applicants.

After completion of the final test, another rating system and vital signs were administered to assess the participants' stress levels. Blood was drawn and blood tests were performed again on the Coulter Max-MTM to assess white blood cell interaction with platelets, and in duplicate on the PFA-100TM, two clinical instruments to assess post-stress platelet function. The data collected was used by Microsoft Excel software to compare baselines (initial or non-stressed) to post-stress samples to assess correlations. It was expected that there would be differences between individuals, as to how they would respond to stress.

It was explained after completion of the TSST that the evaluation was set up in order to control and induce stress in a laboratory setting (Williams & Haggerty, 2004, p. 277). Incentives such as food and a fifty percent discount on massage therapy gift certificates were given for the participants' time. We monitored for increased anxiety and when necessary, followed up to insure their well-being.

The study methods and informed consent document were approved by the Weber State University IRB. An informed consent form was signed and collected from all participants. At no time was subject identity associated with reported findings.

White blood cell and platelet counts were performed on all draws on a Coulter Max-M™ on campus within four hours of collection. Platelet studies were performed on all draws on a PFA-100™ in the clinical laboratory at McKay-Dee Hospital within four hours of collection. Time on both assays was critical to obtaining accurate results. The entire research project was performed by three undergraduate students, who each hold a certification as a medical lab technician and are trained in taking vital signs and performing blood draws as well as laboratory analysis. The project was mentored and supervised by Kara Hansen-Suchy, M.Ed., MT (ASCP) from the Clinical Laboratory Sciences department at Weber State University. This research project

was performed due to the lack of inconclusive research regarding the effects of mental stress on platelet function and clotting mechanisms.

Results

We found that after administration of the Trier Social Stress Tests (TSST), the means of systolic blood pressure increased by an average of two mm Hg, diastolic blood pressure increased by an average of five mm Hg, and pulses increased by seven beats per minute from prestress baseline levels. The questionnaires showed the average stress rating increased by one level. Platelet function analysis decreased by an average of three seconds in relation to the baseline. The results of the stress ratings, vital signs, and platelet function analysis are shown in Table I.

The means of the post stress platelet counts, total white blood cell counts, absolute lymphocyte, and absolute monocyte counts were compared to pre-stress baselines. The average platelet counts decreased by 4,000 cells/ μ L, and the total white blood cell count decreased by 200 cells/ μ L. The other white blood cell parameters are as follows; the absolute lymphocyte count decreased by 100 cells/ μ L and there was no change in the absolute monocyte count. The results of the platelet count and white blood cell parameters are in Table 1.

T 1 1 4	· · ·	1		1 1	11 1	1 1
Table 1	(ampartean of	nre- and	post-stress vita	Leigne and	hlood	camples
Iable I	COMPANISON OF	DIC and	DUST SITUSS VIIA	i signs and	DIOUC	i samuutes.

Baseline	Post Stress	t-Test p(0.05)
5	6	0.337
74	81	0.018*
121	123	0.208
75	80	0.105
122	119	0.402
292	288	0.112
7.3	7.1	0.091
2.3	2.2	0.036*
0.5	0.5	0.431
	5 74 121 75 122 292 7.3 2.3	5 6 74 81 121 123 75 80 122 119 292 288 7.3 7.1 2.3 2.2

^{*} Indicates significance at this alpha level.

Discussion

Although there was a slight decrease between the baseline and post-stress means of the platelet function analysis, the difference was not statistically significant at p \leq 0.05. Baseline and post-stress data of all parameters including platelet function analysis, stress ratings, vital signs, platelet counts, and white blood cell parameters were analyzed using a paired t-test with an alpha level of 0.5. There was a statistical significance in the change of the pulses and the absolute lymphocyte counts.

Caution must be used in detecting the slight decrease in platelet function between the baseline and post-stress data due to the high variance. Therefore, data remains inconclusive in support of the hypothesis of increased platelet function after stress stimulation. The counts in platelet and white blood cells appeared to be slightly decreased, but this change is also statistically insignificant. The difference between the absolute lymphocyte counts from pre- to post-stress stimulation appears to be statistically significant; however, data remains uncertain and cannot be linked directly to platelet function.

The data remains inconclusive and we cannot accept or reject our hypothesis based on the current analysis. This was due to the small sample size and possible inadequate stress induction in the subjects. Further research will need to be performed in order to look into these slight changes of platelet function and blood cell analysis, to conclude if there could be significance to these findings.

Suggestions for continued research include using a larger sample size, increase the mental stress factor, and use of both epinephrine and ADP PFA- 100^{TM} cartridges. In this study, only the epinephrine collagen cartridges were used due to time constraints.

Acknowledgments

The authors wish to express their appreciation to Dade Behring for supplying reagents at reduced price, and Intermountain Health Care and McKay-Dee Hospital for providing use of their PFA-100™ analyzer. Also, to the Office of Undergraduate Research at Weber State University for supplying the funds for the research, the Psychology department, and the Clinical Laboratory Sciences department for their expertise, support and help.

References

- Aurigemma, C., Infusino, F., Crea, F., & Sestito, A., (2005). Platelet reactivity in response to mental stress in syndrome X and in stable or unstable coronary artery disease. *Thrombosis research*, 116(1), 25.
- Brydon, L., Magid, K., & Steptoe, A. (2006). Platelets, coronary heart disease, and stress. *Brain, behavior, and immunity* 20, 113.
- Erusalimsky, J., & Edwards, B. L. (2003). The influence of psychological stress and socioeconomic status on platelet activation in men, *Atherosclerosis*, 168(1), 57.
- Gibson, L., Hamer, M., Steptoe, A., et al. (2006). Inflammatory and hemostatic responses to repeated mental stress; Individual stability and habituation over time. *Brain, Behavior, and Immunity*, 20(5), 456.
- Lederbogen, F., Reka, B., & Maria, G. (2004). Effect of mental and physical stress on platelet activation markers in depressed patients and healthy subjects: a pilot study. *Psychiatry Research*, 127, 55.
- Mammen, E., Comp, C. P., & Gosselin, R. (1998). PFA-100™ System: A New Method for Assessment of Platelet Dysfunction. *Seminars in Thrombosis and Hemostasis*, 24(2), 17.
- Strike, P., Kesson, M., & Daisy, W. (2006). Pathophysiological processes underlying emotional triggering of acute cardiac events. *Proceedings of the National Academy of Sciences of the United States of America*, 103(11), 4322.
- Williams, R. A., & Hagerty, B. M. (2004). Trier Social Stress Test; A Method for Use In Nursing Research. *Nursing Research*, 53(4), 277.

MICROBIOLOGY

Isolation and Characterization of Chitin-Utilizing Halophiles from the Great Salt Lake, Utah

Kevin M. Bowcutt, Brigham Burton & Dan R. Cox*

Faculty Mentors: Michele D. Zwolinski & Craig J. Oberg

Abstract

The Great Salt Lake's south arm contains a large biomass of brine flies and shrimp whose husks and exoskeletons are composed of chitin, a recalcitrant carbon source. Isolation procedures were used to find halophilic microorganisms capable of degrading chitin. Samples obtained from Bridger Bay off Antelope Island were inoculated into enrichment media selective for chitinolytic halobacteria. Enrichment flasks were incubated at 22°C in a shaker for three weeks. A selective chitin agar was then developed to ensure that the chitin flakes would stay near the surface of the agar by allowing a layer of minimal halophilic agar to solidify and then pouring 10 mL of chitin agar as an overlay. Fourteen different isolates capable of growing on chitin were obtained from the enrichment cultures. Most isolates were obtained from exoskeleton debris with the majority Gram-negative rods or cocci. Nearly half displayed some pigmentation. Based on their 16S rRNA gene sequences, the isolates are related to common halophilic organisms including the Gram negative Halomonas sp. and Salinivibrio costicola, and Gram positive Marinococcus sp., Bacillus baekryungensis, Salinicoccus roseus and Brachybacterium sp. A chitinase assay is currently under development to quantify this activity among the isolates. The chitinase activity of these halophilic microorganisms could be utilized in insecticide and biodegradation applications.

Introduction

The Great Salt Lake (GSL), located in the arid Great Basin, is a remnant of the prehistoric freshwater lake, Lake Bonneville. Today, this terminal lake exists as a dichotomous ecosystem, with a north arm and a south arm divided by a rock railroad causeway built in 1959 (Post, 1977). The sodium chloride (NaCl) concentration of the north

arm is near saturation, whereas the south arm is much lower, averaging approximately 12% NaCl (Ollivier et al., 1994). Only a few types of microorganisms are capable of living in this hypersaline ecosystem, including eukaryotic algae such as Dunaliella salina, the bacterial families Halobacteriaceae and Haloanaerobiacea, and some cyanobacteria like Oscillatoria spp. (Ollivier et al., 1994). In addition to bacteria, halophilic archaea are found in the GSL, with their concentration more pronounced in the north arm (Baxter, 2005). In hypersaline environments, species diversity appears to decrease as salinity increases (Post, 1977).

The only significant macroscopic life found abundantly in this hypersaline environment are brine shrimp (Artemia salina) and brine flies larvae (Ephydra hians and E. gracillis). Dense populations of brine shrimp and brine flies are responsible for the deposition of chitin and other organic matter in these hypersaline environments (Liaw & Mah, 1992). This large biomass of brine fly husks and dead brine shrimp can be a recalcitrant carbon and nitrogen source, resulting in immense deposits of organic matter along the shoreline due to wind and wave action.

Chitin is composed of repeating units of the monomer N-acetyl-D-glucosamine and is the second most abundant biopolymer found in nature. In aquatic systems alone, chitin production has been estimated at 10¹¹ metric tons per year (LeCleir et al., 2004). In marine environments, degradation of chitin by marine chitinolytic bacteria is essential in the recycling of carbon, nitrogen, and energy. Breakdown and biotransformation of marine organic molecules, such as chitin, is mediated primarily by bacterial communities via catabolic enzyme systems (Ramaiah et al., 2000). Chitinases are enzymes that hydrolyze the \beta1-4 glycosidic bonds that link the N-acetyl-D-glucosamine molecules which compose chitin (Metcalfe et al., 2002). This hydrolysis typically yields oligomeric or dimeric residues capable of being transported across the cellular membrane where further metabolism occurs. Chitinases are often associated with the outer membrane of the cell or can be secreted as extracellular enzymes (LeCleir et al., 2004). Chitinases are classified either as family 18 or 19 glycosyl hydrolases based on their amino acid sequence similarity. The vast majority of character-

^{*} Weber State University Undergraduate Research Scholarship Award

ized bacterial chitinases fall within family 18 which is further divided into five different groups (I to V) based on conservation of amino acid residues within the catalytic domain (LeCleir et al., 2004).

Some ecological studies have investigated the prevalence of chitinolytic bacteria and rates of chitin degradation in the environment; however, there have been very few studies of chitinolytic bacteria in hypersaline environments such as the GSL (Ramaiah et al., 2000). In the present study, attempts were made to isolate and characterize halophilic chitin-digesting microorganisms from the GSL in order to verify which, if any, halophilic microorganisms utilize chitin as a carbon source and to determine how widespread this metabolic trait may be among halophiles.

Methods

Site Description and Sampling

Samples from the south arm of the GSL were collected along the northwest shore of Antelope Island near Bridger Bay. Sediment and water samples were collected 10-15 meters from the shoreline, and samples of suspended exoskeletons were collected within five meters of the shoreline. The sampling dates and sources are listed in Table 1.

Media

Halobacteria Medium was prepared using the following formulation (NaCl, 80 g; agar, 10 g; MgSO₄·7H₂O, 10 g; casein hydrolysate, 5 g; KCl, 5 g; trisodium citrate, 3 g; KNO₃, 1 g; yeast extract, 1 g; CaCl₂·6H₂O, 0.2 g per liter) with trisodium citrate substituted for disodium citrate (Atlas, 1993). A halobacteria broth was prepared using this same formulation without addition of the agar. Chitin agar was prepared using the following formulation (agar, 15 g; precipitated chitin, 4 g; (NH₄)₂SO₄, 2 g; Na₂HPO₄, 1.1 g; KH₂PO₄, 0.7 g; MgSO₄·7H₂O, 0.2 g; FeSO₄, 1.0 mg; MnSO4, 1.0 mg per liter) modified to 8% NaCl (Atlas, 1993). Crabshell chitin (C7170, Sigma Chemical, Dallas, TX) was purified prior to addition to the chitin agar. The chitin was then washed by the addition of ten grams of chitin to 100 mL of concentrated HCl in a 250 mL flask, placed on a shaker for one hour, and then centrifuged at 2500 rpm for 10 min. Following the acid wash, the chitin was washed with 100 mL

of distilled water and centrifuged as previously described. This wash process was repeated until the suspension reached an approximate pH of 7 (approximately 10 wash cycles). The final pH was then adjusted to 7 using 1 N KOH. A two-step pour plate technique was utilized to provide a layer of chitin nearer to the inoculum. This was done by pouring a 20 mL base layer of chitin agar without chitin into each Petri plate, then overlaying the solidified base agar with 10 mL of the chitin agar containing 0.4 g of purified chitin per 100 mL of media.

Enrichment and Isolation Procedures

An enrichment technique was utilized to select for halophilic bacteria capable of chitin degradation. The enrichment broth was made by using the Halobacteria Medium minus agar, yeast extract, and casein hydrolysate. Chitin was added to the medium at a concentration of 2.5 g/L. Individual one mL samples from the various GSL sampling locations, were placed in sterile 250 mL screw cap flasks containing 150 mL of enrichment broth. Samples were incubated in a shaker at 22°C for three weeks (Figure 1). Chitin was the sole carbon source in the enrichment media.

Chitin agar Petri plates were inoculated with the biofilm located at the media interface in the enrichment flasks to isolate chitin-degrading organisms. Plates were incubated at 22°C for 14-21 d. Individual colonies were picked off the plates with a sterile loop and streaked for isolation on fresh chitin agar plates. Once pure cultures were obtained they were grown in Halobacteria Medium broth until turbid and stored at 5°C until needed for further characterization. Individual isolates were Gram stained to assure purity prior to 16S rRNA analysis.

DNA Extraction, 16S rRNA Gene Amplification and Sequencing

DNA was extracted from the isolates using a modified bead beater-lysis method with phenol-chloroform purification. Isolation of the DNA was confirmed on a 1% agarose gel. The 16S rRNA gene was amplified using bacteria specific primers (27F 5' AGA GTT TGA TCM TGG CTC AG 3' / 1492R 5' ACG GYT ACC TTG TTA CGA CTT 3') (Lane, 1991). The reaction mixture contained 200 nM of each primer, 250 μ m of the dNTPs, 0.2 mg/mL bovine serum albumin, 1U DNA Tag Poly-

merase and the diluted reaction buffer (Promega, Madison, WI). The amplification parameters were 94°C for 3 min., followed by 25 cycles of 94°C for 45 s, 57°C for 1 min., 72°C for 2 min., and a final extension step at 72°C for 7 min. Approximately 659 base pairs were sequenced (Genewiz, Inc., NJ). The sequences were then compared to the Gen-Bank database using the BLAST search tool.







#5 No disodium citrate

#5 With disodium citrate

#1 No disodium citrate

Figure 1 Enrichment cultures for the isolation of chitin utilizing microorganisms from the Great Salt Lake.

Results

Enrichment flasks yielded observable biofilms attached to the flask wall at the media surface after 10 d of incubation. Many of the biofilms contained pigmented organisms yet no observable pigmentation was observed in the original samples obtained from the GSL (Figure 1).

Fourteen different isolates were obtained using the enrichment and selective chitin agar techniques (Figures 2 and 3). Table 2 displays the physiological traits of each of the fourteen isolates. In addition to growing on the chitin agar, most of the isolates produced an observable zone of clearing around the colony indicating the ability to degrade chitin. Isolation of different pigmented colonies was done to obtain a spectrum of bacterial types.

Based on their 16S rRNA gene sequences, the isolates are related to common halophilic organisms including Gram negative *Halomonas*

sp. and Salinivibrio costicola, and Gram positive Marinococcus sp., Bacillus backryungensis, Salinicoccus sp. and Brachybacterium sp. Table 3 displays the results of the 16S rRNA amplification and sequencing, including the best match and percent similarity. The Halomonas strains were closely related but their colony morphologies were quite different.

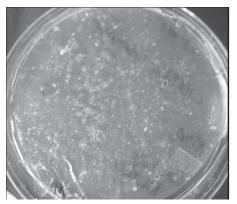




Figure 2 Chitin agar with chitin utilizing organisms.



Figure 3 Selected chitin utilizing isolates.

Discussion

This paper is the first report of bacterial chitinolytic survey data from a hypersaline environment. Only a few of life forms inhabit hypersaline ecosystems, although a wide range of substances are available. A large proportion of the available carbon in the GSL ecosystem is bound up in the exoskeletons of brine shrimp and larval husks of brine flies in the form of chitin (Tsai et al., 1995). Yu et al. (1991) noted that

the oceans would be depleted of carbon and nitrogen in a relatively short time if chitin could not be returned to the ecosystem in a biologically utilizable form. This carbon source has not been reported to be a significant source for halophiles, however our study showed that there are a variety of microorganisms in the GSL able to degrade and use chitin as a carbon source. Fourteen different chitin-utilizing isolates were obtained using the enrichment technique and selective chitin agar method representing six different genera of halophilic bacteria. Hobel et al. (2005) found chitinase gene diversity was at least two-fold higher in enrichment samples than in controls, indicating a much higher diversity of hydrolytic genes can be obtained with this approach which may explain the broad range of represented genera.

The majority of the isolates were obtained from the suspended exoskeleton debris located along the shore. Cottrell et al. (1999) found a much higher percentage of marine bacteria chitinase producers among estuary isolates than isolates from coastal waters. Bassler et al. (1991) observed that marine bacteria would "sense" and then adhere to chitin oligosaccharides before diffusion and water currents could deprive the bacteria of these potential nutrients. Both observations are consistent with our findings that halophilic chitin degraders were along the shore where chitin sources are deposited.

Nearly half of the halophilic chitin-degrading isolates displayed pigmentation. This coloration may be a mechanism to prevent UV photodamage by absorbing harmful UV radiation (Ehling-Schulz et al., 1997). In addition, the enrichments were done in a shaker chamber containing a window allowing constant light exposure to the enrichment flasks. Since many halophiles have pigmented proteins known as proteorhodopsins (Mongodin et al., 2005) this may have influenced the selection of isolates. Also, most of the isolates were Gram negative rods, an observation consistent with bacterial surveys of marine waters. Three of the isolates were Gram positive, a finding consistent with the observation of Giovannoni and Rappe (2000) who found that Gram positive bacteria are quite rare in seawater, another NaCl environment. Although fewer in overall numbers, Sanchez-Porro et al. (2003) found that Gram-positive moderately halophilic bacteria showed more hydrolytic activities than their Gram-negative counterparts.

Table 1 Sources and dates when isolates were obtained.

Isolate	Sample Source	Enrichment Sample	Date Obtained
LSBB	water	NA	6/28/06
LOM	water	#1 disodium citrate	10/26/06
YMBB	sediment	NA	6/28/06
BMBB	sediment	NA	6/28/06
OMBB	sediment	NA	6/28/06
SBB	sediment	NA	12/6/06
CMBB	sediment	NA	11/20/06
PSBB	sediment	NA	6/28/06
CMW	suspended debris	#5 disodium citrate	11/15/06
SM	suspended debris	#5 chitin sample	11/10/06
WM	suspended debris	#5 chitin sample	11/10/06
WGS	suspended debris	#5 digested brine	11/5/06
TT	suspended debris	#5 biofilm	10/26/06
SMW	suspended debris	#5 chitin sample	11/20/06

Table 2 Physiological traits of isolates.

Isolate	Gm -/+	Morphology	Colony Color
LSBB	-	Bacillus	Burnt Orange
LOM	+	Bacillus	Light Orange
YMBB	-	Coccus	Yellow
BMBB	-	Coccus	Brown
OMBB	+	Coccus	Orange
SBB	+	Coccus	Salmon
CMBB	-	Bacillus	White
PSBB	-	Bacillus	Peach
CMW	-	Bacillus	White
SM	-	Bacillus	White
WM	-	Bacillus	White
WGS	-	Bacillus	White/Green Sheen
TT	-	Bacillus	Tan
SMW	-	Bacillus	White

The isolates described belong to genera commonly isolated from the GSL, indicating that the ability to degrade and use chitin may be prevalent among these halophiles although there have not been any chitinolytic surveys done of moderate halophiles. Surveys of chitinolytic activity among marine bacteria suggest that approximately 10% of culturable bacteria degrade chitin (Okutani, 1975) while the number of marine strains that can utilize the monomer, N-acetyl-D-glucosamine, may be as high as 90% (Martinez et al., 1996). Okamoto et al. (2004) performed a comparative phylogenetic analysis of euryhaline halophiles (bacteria that can grow in environments with widely variable salinities) and found only two strains of Halomonas variabilis out of 10 that could metabolize N-acetyl-D-glucosamine as the sole single carbon source. Since there is a high concentration of chitin in the GSL due to its unique macroscopic life this may select for halophilic microorganisms that utilize chitin when compared to halophiles from other environments. Gene analysis of the halophile Salinibacter ruber genome revealed it contained a full complement of fermentative genes for the degradation and transport of organic compounds including a

Table 3 Analysis of 16S rRNA sequences for chitinolytic isolates.

Isolate	Best Match	Accession #	% Similarity
LSBB	Halomonas ventosae	AY268080	100%
LOM	Bacillus baekryungensis strain G SP 53	AY505506	99%
YMBB	Brachybacterium sp. B-4051	DQ347546	99%
BMBB	Halomonas sp. AJ275	EF144149	99%
OMBB	Marinococcus sp. HS209	DQ458831	98%
SBB	Salinicoccus sp. D23.3	AJ717730	99%
CMBB	V. costicola (ATCC 35508T)	X74699	99%
PSBB	Halomonas sp. LCKS0-Isolate 1	DQ395131	99%
CMW	Halomonas sp. LCKS0-Isolate 1	DQ395131	100%
SM	Halomonas sp. LCKS0-Isolate 1	DQ395131	99%
WM	Halomonas sp. LCKS0-Isolate 1	DQ395131	100%
WGS	Halomonas sp. LCKS0-Isolate 1	DQ395131	99%
TT	Salinivibrio costicola strain GSP14	AY553070	99%
SMW	Salinivibrio costicola strain GSP14	AY553070	99%

chitinase gene (Mongodin et al., 2005) although its ability to express this trait was not determined.

The chitinase activity displayed by halophilic bacteria in the GSL could prove to be useful in industry. Exoskeletons of insects are composed of chitin, and there is the possibility that chitinase could be used as an effective insecticide. Chen et al. (1991) found a high correlation between chitinolysis and the production of other bioactive compounds in bacteria indicating this type of enrichment and screening could be used as a preliminary step in the bioprospecting of halophilic microorganisms for metabolic compounds useful in the medicine, bioremediation, and industrial processes.

References

- Atlas, R. M. (1993). *Handbook of Microbiological Media*. Parks, L. C. (ed.) CRC Press, Boca Raton, FL.
- Bassler, B. L., Yu, C., Lee, Y. C., & Roseman, S. (1991). Chitin utilization by marine bacteria: Degradation and catabolism of chitin oligosaccharides by *Vibrio furnissii*. *Journal of Biological Chemistry*, 36, 24276-24286.
- Baxter, B. K., Litchfield, C. D., Sowers, K., Griffith, J. D., Dassarma, P. A., & Dassarma, S. (2005). Microbial diversity of GSL. In *Adaptation to life at high salt concentrations in Archaea, bacteria, and eukarya.* Gunde-Cimerman, N., Oren, A., and Plemenitas, A. (ed.) Springer, Netherlands.
- Cottrell, M. T., Moore, J. A., & Kirchman, D. L. (1999). Chitinases from uncultured marine microorganisms. *Applied and Environmental Microbiology*, 65, 2553-2557.
- Ehling-Schulz, M., Bigler, W., & Scherer, S. (1997). UV-B-induced synthesis of photoprotective pigments and extracellular polysaccharides in the terrestrial cyanobacterium. *Nostoc commune. Journal of Bacteriology*, 179, 1940-1945.
- Giovannoni, S., & Rappe, M. (2000). Evolution, diversity, and molecular ecology of marine prokaryotes, p. 47-84 In D. L. Kirchman (ed.) *Microbial ecology of the oceans*. John Wiley and Sons Inc., New York, NY.
- Hobel, C. F. V., Marteinsson, V. T., Hreggvidsson, G. O., & Kristjansson, J. K. (2005). Investigation of the microbial ecology of intertidal hot springs by using diversity analysis of 16S rRNA and chitinase genes. *Applied and Environmental Microbiology*, 71, 2771-2776.
- Lane, D. J. (1991). 16S/23S rRNA sequencing. In Stackebrandt E., & Goodfel-

55 ◆ Ergo

- low, M. (eds), Nucleic Acid Techniques in Bacterial Systematics. p. 115-175.
- LeCleir, G. R., Buchan, A., & Hollibaugh, J. T. (2004). Chitinase gene sequences retrieved from diverse aquatic habitats reveal environment-specific distributions. *Applied and Environmental Microbiology*, 70, 6977-6983.
- Liaw, H. J., &Mah, R. A. (1992). Isolation and characterization of Haloanaerobacter chitinovorans gen. nov., sp. nov., a halophilic, anaerobic, chitinolytic bacterium from a solar saltern. Applied and Environmental Microbiology, 58, 260-266.
- Martinez, J., Smith, D. C., Stewart, G. F., & Azam, F. (1996). Variability in ectohydrolytic enzyme activities of pelagic marine bacteria and its significance for substrate processing in the sea. *Aquatic. Microbiology. Ecology.*, 10, 223-230.
- Metcalfe, A. C., Krset, M., Gooday, G. W., Prosser, J. I., & Wellington, E. M. H. (2002). Molecular analysis of a bacterial chitinolytic community in an upland pasture. *Applied and Environmental Microbiology*, 68, 5042-5050.
- Mongodin, E. F., Nelson, K. E., Daugherty, S., DeBoy, R. T., Wisner, J., Khouri, H., Weidman, J., Walsh, D. A., Papke, R. T., Sanchez Perez, G., Sharma, A. K., Nesbo, C. L., MacLeod, D., Bapteste, E., Doolittle, W. F., Charlebois, R. L., Legault, B., & Rodriguez-Valera, F. (2005). The genome of Salinibacter ruber: Convergence and gene exchange among hyperhalophilic bacteria and archaea. Proceedings of the National Academy of Sciences, 102(50), 18147-18152.
- Okamoto, T., Maruyama, A., Imura, S., Takeyama, H., & Naganuma, T. (2004). Comparative phylogenetic analyses of *Halomonas varibilis* and related organisms based on 16S rRNA, gyrB and ectBC gene sequences. *Systematic and Applied Microbiology*, 27, 323-333.
- Okutani, K. (ed.) (1975). *Microorganisms related to mineralization of chitin in aquatic environments*. University of Tokyo Press, Tokyo, Japan.
- Ollivier, B., Caumette, P., Garcia, J. L., & Mah, R. A. (1994). Anaerobic bacteria from hypersaline environments. *Microbiological Reviews*, 58, 27-38.
- Post, F. J. (1977). The microbial ecology of the GSL. Microbial Ecology, 3, 143-165.
- Ramaiah, N., Hill, R. T., Chun, J., Ravel, J., Matte, M. H., Strauge, W. L., & Colwell, R. R. (2000). Use of a *chiA* probe for detection of chitinase genes in bacteria from the Chesapeake Bay. *FEMS Microbiology Ecology*, *34*, 63-71.
- Sanchez-Porro, C., Martin, S., Mellado, E., & Ventosa, A. (2003). Diversity of moderately halophilic bacteria producing extracellular hydrolytic enzymes. *Journal of Applied Microbiology*, 94, 295-300.

- Tsai, C. R., Garcia, J. L., Patel, B. C., Cayol, J. L., Baresi, L., & Mah, R.A. (1995). *Haloanaerobium alcaliphilum* sp. nov., an anaerobic moderate halophile from the sediments of GSL, Utah. *International Journal of Systematic Bacteriology*, 45, 301-307.
- Yu, C., Lee, A. M., Bassler, B. L., & Roseman, S. (1991). Chitin utilization by marine bacteria. *Journal of Biological Chemistry*, 266, 24260-24266.

MICROBIOLOGY

Inhibition of Common Spoilage Fungi by Lactic Acid Bacteria

Karli E. Oberg, Miriam N. Bernardo & Kris Froerer

Faculty Mentors: Matthew J. Domek & Craig J. Oberg

Abstract

Fungal spoilage of perishable foods, including dairy products, is a significant problem with few remedies since any compound utilized must be safe for human consumption. Lactic acid bacteria (LAB) can inhibit other types of microorganisms by producing organic acids, peroxidases, or bacteriocins. LAB strains were screened for fungal inhibition against Penicillium notatum, Aspergillus niger, and Rhizopus stolinifer using the agar flip protocol. A large colony from each of 25 LAB (Lactococcus, Lactobacillus, Streptococcus, and Leuconostoc species) was inoculated onto the center of a MRS agar plate. Following 48 h, the agar was aseptically flipped over and the test fungus was swabbed on the exposed surface. Plates were incubated at 25°C with inhibition monitored over 72 h. Eighteen of 25 LAB tested showed inhibition against multiple fungal strains. A second screening test utilizing these 18 LABs was done to see if it could be determined whether the inhibition was due to primary metabolites (24 h colonies) or, perhaps, secondary metabolites (72 h colonies). While some fungal inhibition by the 24 h old LAB colonies was observed, there was significantly more inhibition of all test fungi when the LAB colonies were challenged following 72 h of incubation. Rhizopus was the most resistant fungus while Penicillium was the most susceptible to LAB inhibition. All Leuconostoc strains tested showed inhibition, perhaps because they are heterofermentative and produce a variety of organic acids. A number of Lactobacillus species, particularly L. helveticus strains, were inhibitory. Results suggest some LAB strains could be producing anti-fungal compounds, particularly since fungal inhibition was observed when plates were incubated out to 21 d. Very few homofermentative Lactococcus and Streptococcus strains were inhibitory. LAB that inhibit spoilage fungi may have application, either incorporated in a product like cheese or sprayed on a product, to increase its shelf life.

Introduction

Mold spoilage of foods, particularly perishable foods like refrigerated and fermented dairy products, cost the food industry and consumers millions of dollars each year in the Unites States (Frank, 2001). Fungal growth can spoil fermented dairy products following the opening of vacuum-sealed packaging, which allows oxygen in the package, even when the product is held under refrigeration temperatures (Marth, 1978). Very few naturally produced antifungal products are currently available for use in or on perishable foods. A number of organic acids are used in beverages and in baked goods to suppress fungal growth but over time at refrigerated storage temperatures even these prove ineffective (Frank, 2001). If strains of LAB from dairy sources or even their metabolites could be incorporated into these types of products, shelf life could be increased and the volume of product lost to spoilage decreased.

A variety of inhibitory mechanisms are utilized by strains of lactobacilli to inhibit fungi. Corsetti et al. (1998) found that obligately heterofermentative strains of Lactobacillus inhibited Penicillium, Fusarium, Aspergillus, and Monilia molds by producing a mixture of organic acids. Included in this mixture were acetic, caproic, formic, propionic, butyric, and n-valeric acids, many of which appeared to work in a synergistic manner. A comparison of individual acids indicated caproic acid played a key role in inhibiting mold growth. Florianowicz (2001) showed that strains of Lactobacillus casei, Lactobacillus delbrueckii ssp. bulgaricus, and Lactobacillus lactis ssp. cremoris were active inhibitors of Penicillium expansum in a direct contact assay. Stiles et al. (1999) examined antifungal activity by 11 strains of lactobacilli and found that two strains of Lactobacillus casei var. rhamnosus and a Lactobacillus salivarius could significantly suppress mold growth. In addition, cell free extracts, either heated or acid neutralized, also inhibited the growth of Fusarium sp. DMF 0101 indicating that other antifungal substances were being produced besides organic acids and hydrogen peroxide. Lavermiocca et al. (2000) found two acids, phenyllactic and 4-hydroxy-phenyllactic, inhibited a broad spectrum of fungi including Penicillium and Aspergillus species. Karunaratne et al. (1990) found that the living cells from three species of lactobacilli (Lactobacillus acidophilus, L. delbrueckii ssp. bulgaricus, and L. plantarum) were effective in preventing growth of Aspergillus and that their bacterial metabolites were effective in reducing the amount of aflatoxin produced although overall mold growth was not inhibited. In this study prevention of mold growth was determined to be primarily due to a pH effect and microbial competition.

Production of very specific anti-fungal compounds has also been investigated in LAB. In a large screening study, Gourama (1997) screened 420 LAB isolates from dairy, vegetables and fruits for antifungal activity and found four to be inhibitory to four Penicillium species. Two of the inhibitory isolates were Lactobacillus casei strains and their inhibitory activity was shown to be unrelated to lactic acid or hydrogen peroxide production and sensitive to proteolytic enzymes indicating the production of an antifungal protein. A number of metabolites from various Lactobacillus cultures have been suggested to have antifungal properties (Plockova et al., 1997). Okkers et al. (1999) showed that TV35b, a bacteriocin-like protein isolated from a Lactobacillus pentosus strain, also inhibited Candida albicans along with a number of lactotbacilli and clostridial species. An antifungal substance is also produced by Lactobacillus acidophilus R grown in Ellikers broth (Batish et al., 1999). Gourama and Bullerman (1997) found that L. casei var. pseudoplantarum 371 isolated from a silage inoculant could inhibit aflatoxin biosynthesis by Aspergillus when the two organisms were grown in a liquid medium but that the Lactobacillus had no effect on aflatoxin production or mold growth when inoculated together on a solid medium. Supernatant liquid was inhibitory of production of aflatoxins B and C without affecting mold growth. Magnusson and Schnurer (2001) isolated a broad-spectrum proteinaceous antifungal compound from a Lactobacillus. Strom et al. (2002) isolated two antifungal cyclic dipeptides from Lactobacillus planterum which are able to inhibit Aspergillus fumigatus. These studies suggest similar antifungal compounds may be produced by dairy LAB, particularly strains of Lactobacillus and Leuconostoc.

In this project, LAB isolates from dairy sources were screened for fungal inhibition to identify those LAB strains that might produce specific antifungal compounds.

Methods

Isolation of Probiotic Bacteria

Strains of dairy LAB were isolated from a variety of commercial sources or obtained from ATCC (Manassas, VA, USA) (Table 1). When

required, genus and species designations were confirmed using gram stain and API sugar panels (API 50 CHL, bioMerleuz, Haywood, MO). LAB cultures were all grown in MRS broth (Becton, Dickinson, and Co., Sparks, MD). After the MRS broth was inoculated, tubes were incubated at 37°C for 24 hours and then refrigerated for a two-week period. After two weeks of refrigerated storage, the bacteria had to be transferred to sterile MRS broth and incubated as described above. Each culture was streaked for isolation on an MRS plate to confirm purity before inhibition tests were performed. Fungi (molds) used included Penicillium notatum (ATCC 11625), Aspergillus niger (ATCC 16880), and Rhizopus stolinifer (ATCC 13963). These mold strains were selected as model organisms because of their potential to cause food spoilage. Molds were grown on Sabouraud agar plates (Difco Laboratories, Detroit, MI) for seven days at room temperature. To prepare the fungal inoculums, two Sabouraud plates were spread with each mold and incubated at 25°C until a confluent lawn containing a significant number of spores was present (approximately 96 hours). The 0.5 McFarland standard was used to quantify spore suspensions for the inhibition test. Fungal spores were harvested from the mold lawn on the plate with a sterile cotton swab and put into sterile 0.85% saline until this concentration was achieved.

Fungal Inhibition Screening

A test run was performed utilizing TSA media and MRS media. Both media were spread with each test mold and incubated for 48 hours at 30° C to determine which agar would support fungal growth. The MRS agar had better mold growth on it, so this medium was used for the rest of the experimental procedures contrary to the findings of Stiles et al. (2002) who reported that MRS was inhibitory to fungi due to the presence of sodium acetate in the medium. Individual LAB cultures were grown in MRS broth for 24 hours at either 30° C or 37° C depending on the culture's optimal growth temperature. Each MRS plate was spotted with 30 microliters (μ L) of an LAB (approximately 1 X 10^{8} CFU per mL). Inoculated plates were incubated for 48 hours at 37° C in Gaspaks. Following incubation, a large colony formed in the center of each petri dish. Then, using the "agar flip"

61 • Ergo Featured Articles • 62

method, the agar was aseptically flipped over into the lid of the petri dish. To perform this technique, a spatula was dipped into 70% ethanol, flamed, and then used to carefully flip the inoculated agar slab into the petri dish lid. On the exposed side, one of the three fungal spore suspensions was swabbed across the agar surface using a sterile cotton swab. Screening plates were incubated aerobically for 48 hours at 30°C. Zones of inhibition were measured across the entire zone of observable clearing then grouped into a four plus scale where = no inhibition, + = 1-20 mm of clearing, ++ = 21-40 mm of clearing, +++ = 41-60 mm, and ++++ = > 60 mm of clearing. This measurement was used to determine the antifungal capability of each LAB isolate. LAB isolates that showed the greatest degree and range of inhibition were selected for a second inhibition test.

Effect of LAB Incubation Time on Fungal Inhibition

Individual LAB cultures were grown in MRS broth for 24 hours at either 30°C or 37°C depending on the culture's optimal growth temperature. Thirty microliters (μ L) of each LAB was inoculated onto individual petri plates containing MRS agar using a 100 μ L micropipette. Inoculated plates were incubated anaerobically for either 24 or 72 hours at the appropriate temperature in 2.5 L AnaeroGas chamber (Oxoid Ltd., Hampshire England). After incubation, a large colony formed in the center of each petri dish. Using the "agar flip" method, the agar was flipped over into the lid of the petri dish. After the first inhibition screening test, the zones of inhibition were measured at 3 days and 5 days. Zones of inhibition were measured as previously described and grouped into the four plus scale. The zones of inhibition were also measured after 15 days and 21 days of incubation to see which strains of bacteria were still inhibiting growth of the molds.

Results

Fungal Inhibition Screening

Eighteen of 25 LAB tested showed inhibition against multiple fungal strains (Table 2). Most of the LAB that exhibited fungal inhibition produced large zones of inhibition against the test fungi. The least effective LAB were strain designates 8, 14, 17-21 (primarily *Streptococcus*

Table 1 Lactic acid bacteria used in this study.

ID Number	Ganus and enacine	Source
	Genus and species	
1	Lactobacillus helveticus 200A	Commercial
2	Lactobacillus helveticus WH202	Commercial
3	Lactobacillus helveticus JB212	Commercial
4	Lactobacillus helveticus 15009	ATCC
5	Lactobacillus helveticus WH200	Commercial
6	Lactobacillus delbrueckii ssp. bulgaricus Hansen	Commercial
7	Lactobacillus helveticus JB215	Commercial
8	Streptococcus thermophilus WT13	Dairy
9	Lactobacillus helveticus 10705	ATCC
10	Streptococcus thermophilus WT6	Commercial
11	Bifidobacterium bifidum Hansen 104-57	Commercial
12	Lactobacillus delbrueckii ssp. bulgaricus R44	Dairy
13	Lactobacillus helveticus LH100	Dairy
14	Lactobacillus delbrueckii ssp. bulgaricus R8	Commercial
15	Streptococcus thermophilus WT33	Commercial
16	Lactobacillus delbrueckii ssp. bulgaricus R10	Dairy
17	Streptococcus thermophilus DM10	Commercial
18	Streptococcus thermophilus WT3A	Commercial
19	Streptococcus thermophilus WT26	Commercial
20	Lactococcus lactis ssp. lactis 161	Commercial
21	Lactococcus lactis ssp. lactis 77	Commercial
22	Leuconostoc mesenteroides 8293	ATCC
23	Leuconostoc species CAF	Commercial
24	Leuconostoc mesenteroides	ATCC
25	Leuconostoc citreum 160	Commercial

thermophilus and Lactococcus sp.) that were excluded in the second experiment. Many LAB produced pronounced zones of inhibition and the compounds the LAB secreted seem to diffuse effectively through the agar medium.

Effect of LAB Incubation Time on Fungal Inhibition

A second screening test utilizing the 18 LAB strains with the largest

63 • Ergo Featured Articles • 64

Table 2 Initial screening by lactic acid bacteria (LAB) (48-hour colonies) for fungal inhibition.

LAB	Aspergillus	Penicillium	Rhizopus
1	++	+++	++
2	++	+++	++
3	++	+++	+++
4	++	+++	++
5	++	++	+
6	-	+	++
7	++	++	++
8	-	-	-
9	+	++	++
10	+	+	-
11	++	+++	+++
12	++	+++	++
13	++	++	+++
14	++	++	-
15	+	++	+
16	++	+++	++
17	-	-	-
18	-	++	-
19	-	+	-
20	-	-	-
21	-	-	-
22	++	++	+++
23	+++	+++	++
24	+	++	++

- = no inhibition + = 1-20 mm ++ = 21-40 mm +++ = 41-60 mm ++++ = < 60 mm

zones of inhibition from the initial screening was performed to determine if it could be determined whether the inhibition was due to primary metabolites (24-hour colonies) (Table 3) or, perhaps, secondary metabolites (72-hour colonies) (Table 4). In addition, screening plates were read at both 3 days and 5 days of incubation (Table 3 compared to Table 4) to determine if the inhibitory compounds were continuing to be manufactured, were diffusing rapidly or slowly, and if they

Table 3 Fungal inhibition by LAB (24-hour colonies) following either three or five days of incubation.

LAB	Aspergillus		Penic	Penicillium		Rhizopus	
LAD	3 d	5 d	3 d	5 d	3 d	5 d	
1	+	+	++	++	+	-	
2	++	+	++	+	++	-	
3	+	+	++	+	++	-	
4	++	++	++	+	+	-	
5	++	+	++	+	+	-	
6	++	-	++	+	++	-	
7	+	-	+	+	+	-	
9	+	-	+	+	-	-	
10	+	-	+	+	-	-	
11	++	++	+++	++	++	-	
12	++	-	++	+	+	-	
13	++	+	+++	++	+++	-	
15	+	-	++	+	+	-	
16	-	-	++	+	-	-	
22	++	+	++	++	+	-	
23	+	+	++	+	++	-	
24	++	-	+	+	-	-	
25	-	-	+	+	-	-	

- = no inhibition + = 1-20 mm ++ = 21-40 mm +++ = 41-60 mm

have a residual effect over time. While some fungal inhibition by the 24-hour colonies was observed, there was significantly more inhibition of all test fungi when the LAB colonies were challenged following 72 hours of incubation. *Rhizopus* was the most resistant fungus while *Penicillium* was the most susceptible to LAB inhibition. *Rhizopus* was initially inhibited but showed growth after longer incubation (Tables 3 and 4). All *Leuconostoc* strains tested showed inhibition. A number of *Lactobacillus* species, particularly *L. helveticus* strains, were inhibitory.

Many LAB strains appear to inhibit common spoilage fungi probably utilizing the same metabolic products that have been shown to inhibit bacteria, specifically secreted organic acids and hydrogen peroxide. Results from the second inhibition study suggest some LAB strains could be producing anti-fungal compounds, particularly since

65 ◆ Ergo Featured Articles ◆ 66

Table 4 Fungal inhibition by LAB (72 hr colonies) following either three or five days of incubation.

LAB	Aspergillus		Penic	Penicillium		Rhizopus	
LAD	3 d	5 d	3 d	5 d	3 d	5 d	
1	ND	ND	ND	ND	ND	ND	
2	++	+	++++	+++	+	-	
3	+++	+++	+++	+++	++	-	
4	+++	+	++++	+++	+++	+	
5	+++	+	+++	+++	+	-	
6	++	+	++	++	-	-	
7	+++	+	+++	+++	+++	-	
9	ND	ND	ND	ND	ND	ND	
10	++	+	++	++	-	-	
11	+++	++	++++	+++	+++	++	
12	+++	++	+++	+++	+++	+	
13	+++	++	+++	+++	+++	+	
15	++	+	++	+	-	-	
16	+++	++	++	+++	++	-	
22	+++	++	++	+++	++	-	
23	+++	++	+++	+++	+++	+	
24	++	+	++	++	-	-	
25	++	+	+	+	+	+	

- = no inhibition + = 1-20 mm ++ = 21-40 mm +++ = 41-60 mm ++++ = < 60 mm

fungal inhibition was noted out to 21 d of incubation. Very few homofermentative *Lactococcus* and *Streptococcus* strains were inhibitory. The high rate of inhibition by heterofermentative LAB, including *Leuconostoc* strains, suggest a synergistic effect from multiple metabolic compounds may have a more significant inhibitory effect on the test fungi. Studies were all conducted at an elevated temperature (30°C) well above ambient room temperature. If these studies were done at refrigerated temperatures, the length of incubation may be much longer, thus increasing shelf life even more.

Because the LAB and fungal test organisms were not in direct contact, only compounds produced and secreted by individual LAB cultures could cause fungal inhibition. Since most fungi will grow at an acidic pH, it is unlikely that just LAB-produced lactic acid caused

the inhibition. Unlike other organic acids, lactic acid is not generally used as a mold inhibitor in food products. Its only function in foods is to inhibit pH-sensitive spoilage bacteria. More likely, these LAB are producing some other antifungal compound (Moll et al., 1999).

LAB that inhibit spoilage fungi may have application, either incorporated in a product like cheese or sprayed on a product, to increase the shelf life of perishable foods. Many of the dairy LAB strains effectively inhibited the fungal test organisms. Further LAB strain selection and fungal inhibition characterization is now needed, particularly examination of the specific mechanisms of fungal inhibition by specific LAB.

References

- Batish, V. K., Lam, R., & Grover, S. (1990). Studies on environmental and nutritional factors on production of antifungal substance by *Lactobacillus acidophilus* R. *Food Microbiol*, 7, 199-206.
- Corsetti, A., Gobbetti, M., Rossi, J., & Damiani, P. (1998). Antimould activity of sourdough lactic acid bacteria: identification of a mixture of organic acids produced by *Lactobacillus sanfrancisco CB1*. *Appl. Microbiol. Biotechnol*, 50(2), 253-256.
- Florianowicz, T. (2001). Antifungal activity of some microorganisms against *Penicillium expansum*. *European Food Res. Technol*, 212, 282-286.
- Frank, J. F. (2001). Milk and Dairy Products. p. 111-126 In *Food Microbiology Fundamentals and Frontiers* (2nd ed.) M. P. Doyle, L. R. Beuchat, T. J. Montville. ASM Press, Washington, DC.
- Gourama, H., & Bullerman, L. B. (1995). Inhibition of growth and aflatoxin production of *Aspergillus flavus* by *Lactobacillus* species. *J. Food Prot*, 58, 1249-1256.
- Gourama, H. (1997). Inhibition of growth and mycotoxin production of *Penicillium* by *Lactobacillus* species. *Food Sci. Technol*, 30, 279-283.
- Gourama, H., & Bullerman, L. B. (1997). Anti-aflatoxigenic activity of *Lactoba-cillus casei pseudoplantarum*. *Int. J. Food Microbiol*, 34, 131-143.
- Karunaratne, A., Wezenberg, E., & Bullerman, L. B. (1990). Inhibition of mold growth and aflatoxin production by *Lactobacillus* ssp. *J. Food Prot*, 53, 231-236.
- Lavermicocca, P., Valerio, F., Evidente, A., Lazzaroni, S., Corsetti, A., & Gobbetti, M. (2000). Purification and characterization of novel antifungal

- compounds from the sourdough *Lactobacillus plantarum* strain 21B. *Appl. Environ*. *Microbiol*, 66, 4084-4090.
- Magnusson, J., & Schnurer, J. (2001). *Lactobacillus coryniformis* subsp. *coryniformis* strain Si3 produces a broad-spectrum proteinaceous antifungal compound. *Appl. Environ. Microbiol*, 67, 1-5.
- Marth, E. (1978). Dairy Products, p. 145-172, In *Food and Beverage Mycology*. L. R. Beuchat (ed.) AVI Publishing Co., Westport, CT.
- Moll, G. N., Konings, W. N., & Driessen, A. J. M. (1999). Bacteriocins: mechanism of membrane insertion and pore formation. *Antonie van Leeuwenhoek* ,76, 185-199.
- Okkers, D. J., Dicks, L. M. T., Silvester, M., Joubert, J. J., & Odendaal, H. J. (1999). Characterization of pentocin TV35b, a bacteriocin-like peptide isolated from *Lactobacillus pentosus* with a fungistatic effect on *Candida albicans*. *J. Appl. Microbiol*, 87, 726-734.
- Plockova, M., Chumchalova, J., & Tomanova, J. (1997). Antifungal activity of *Lactobacillus acidophilus* CH5 metabolites. *Potravin*, *15*(1), 39-48.
- Stiles, J., Penkar, S., Plockova, M., Chumchalova, J., & Bullerman, L. B. (2002). Antifungal activity of sodium acetate and *Lactobacillus rhamnosus*. *J. Food Prot*, 65, 1188-1191.
- Stiles, J., Plockova, M., Toth, V., & Chumchalova, J. (1999). Inhibition of Fusarium sp. DMF 0101 by Lactobacillus strains grown in MRS and Elliker broths. Advances in Food Sciences, 21(3-4), 117-121.
- Strom, K., Sjogren, J., Broberg, A., & Schurer, J. (2002). *Lactobacillus plantarum* MiLAB 393 produces the antifungal cyclic dipeptides cyclo(L-Phe-L-Pro) and cyclo (L-Phe-trans-4-OH-L-Pro) and 3-phenyllactic acid. *Appl. Environ. Micrbiol*, 68, 4322-4327.

Zoology

Hold 'em or Fold 'em: Hindlimb Flight Posture of Utah Shorebirds

Lani S. Shepard & Joshua C. McFarland*

Faculty Mentor: Ron Meyers

Abstract

Birds utilize one of two hindlimb flight postures during flight: the legs are either held extended with the feet directed toward the tail or held flexed beneath the body. The support of the limbs in the extended position is a postural activity carried out by slow muscle fibers. Eleven muscles from the hindlimbs of three American Avocets (Recurvirostra americana) and three Black-necked Stilts (Himantopus mexicanus) were selected for examination of slow fiber distribution. All muscles contained both slow and fast fibers, likely a result of the versatile actions required of the hindlimb. Five hypotheses are presented to explain the utilization of two different leg positions among avian orders. Hindlimb flight posture may be explained as a dictate of anatomical constraints of the joint or limb, a behavioral component of flight, a result of body thermoregulation or a method of aerodynamic flight control. Finally, hindlimb posture may represent an ancestral trait not necessarily adaptively driven.

Introduction

Birds use one of two hindlimb postures during flight. The legs are either held under the body with the hip, knee and ankle joints flexed, or are trailed behind the body, a posture in which the hip and knee are flexed, but the ankle is extended (Figure 1). Shorebirds, raptors, ducks, geese and parrots fly using the extended posture, whereas perching birds and woodpeckers use the flexed position (Barrett-Hamilton 1903, Townsend 1909, Shepard and Meyers 2006). There has been surprisingly little research investigating the reason a bird uses one or the other posture, but we are in the process of evaluating several hypotheses (Shepard and Meyers 2006, McFarland and Meyers 2008).

The maintenance of limbs in either of these positions represents

^{*} Phyllis Crosby Gardner Undergraduate Research Scholarship

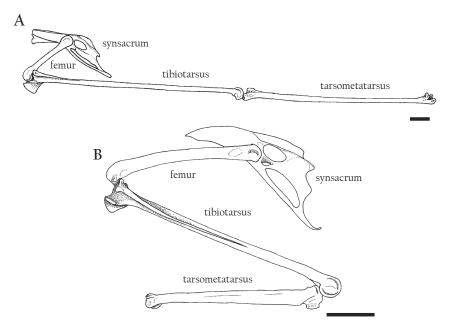


Figure 1 Left lateral view of pelvis and hindlimb illustrating the two hindlimb flight postures. A: Extended posture as seen in the Black-necked Stilt (*Himantopus mexicanus*). B: Flexed posture as illustrated by the Blue Jay (*Cyanocitta cristata*). For clarity, toes are not shown. Scale = 1 cm.

a postural activity. Posture is associated with isometric muscle contractions, which occur when a muscle contracts with no change in length (Goldspink 1980). Slow contracting muscle fibers are considered best suited for isometric contractions, as they are more efficient (Goldspink 1980). Previous research has demonstrated a relationship between a muscle's slow fiber distribution and its function in posture for a number of avian behaviors including folded wing posture (Meyers 1992), drying wing posture in cormorants (Meyers and Mathias 1997), and soaring posture in vultures (Rosser and George 1986), pelicans (Rosser et al., 1994) and albatrosses (Meyers and Stakebake 2005).

We examined two species that use the extended hindlimb posture, American Avocets (*Recurvirostra americana*) and Black-necked Stilts (*Himantopus mexicanus*). These species were chosen due to lack of previous study, extremely long legs, and local availability. Our goals in this study were to (1) document the muscles believed to be involved

in holding the hindlimb in the extended flight posture and quantify their slow, postural muscle fibers, (2) make predictions on the use of these muscles in flight, and (3) discuss the evolutionary implications of these two flight postures in birds.

Methods

After extensive literature review and manipulation of dissected specimens, we chose 11 muscles for study based on their functions as hip flexors, knee flexors, or ankle extensors. Six individuals of American Avocets (*Recurvirostra americana*) and Black-necked Stilts (*Himantopus mexicanus*) were collected locally by shotgun for other projects (US-FWS Permit # MB043593-0; UDWR COR# 1COLL7037). We were given carcasses for dissection within a few hours of death. The left leg and left half of the pelvis were removed intact and stored in phenoxyethanol for later dissection and manipulation. The right leg was dissected and mid-belly sections of each muscle were removed, mounted in 5% gum tragacanth on a cork block, and flash frozen in isopentane cooled to -150°C. These samples were stored at -70°C, then sliced into 12 µm sections using a freezing microtome. The sections were mounted onto microscope slides for tissue reactions.

Muscle sections were reacted with antibodies ALD58 and MY32 to differentiate fiber types (Figure 2). ALD58 reacts positively with slow-contracting muscle fibers, while MY32 reacts positively with fast fibers. After reactions, sections were stained with a commercial streptavadin peroxidase system to mark positively-reacting fibers dark.

Overlapping digital photographs were taken of the reacted muscle, then merged in Adobe Photoshop to create a photo montage illustrating the muscle cross section as a whole. A scale bar was photographed and included with the montage. The image was enlarged and printed to quantify both fast and slow fibers. Three individuals of each species were used for calculating muscle fiber type percentages for all 11 muscles.

For the analysis of hindlimb flight posture across various taxa, we used a variety of databases (e.g., SORA) and extensively searched the literature. We also performed an image search utilizing VIREO, Google Image, and professional photography sites.

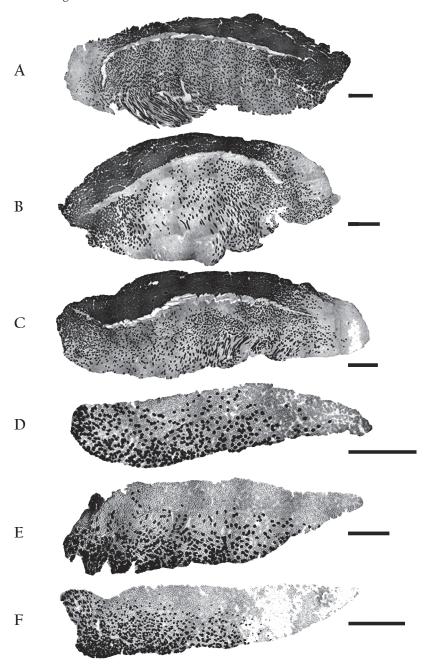


Figure 2 Cross section images of Mm. iliotrochantericus caudalis (A-C) and flexor cruris medialis (D-F) reacted with ALD58. Positively reacting slow fibers appear dark. Images B and D are of *Himantopus*, all others are of *Recurvirostra*. Lateral is to the top of the page, cranial to the right. Scale bars represent 1 mm.

At various aviaries, flying macaws, parrots, and pheasants were photographed with a Canon Digital Rebel XT EOS 8.0 MP SLR camera with a Canon EF 70-300mm f/4-5.6 IS USM telephoto lens. The photographs were analyzed to determine whether flexed or extended posture was present during sustained flight (defined as flight not associated with take-off or landing). We identified a flexed posture if the feet were clearly visible beneath the body. Extended posture was identified if the legs and feet were observed trailing behind the body or facing backward under the tail. If the posture of a bird could not be positively verified, the photograph was not used.

Results

Data for both the avocets and stilts are presented below, organized by muscle functions. Nomenclature follows that of Nomina Anatomica Avium (Baumel et al., 1993).

Hip Flexors

Mm. iliotibialis cranialis, iliotrochantericus caudalis, iliotrochantericus cranialis, and iliotrochantericus medialis support the flexed position of the hip. These muscles each originate on the synsacrum, and insert onto the proximal of the femur. All four muscles contained both slow and fast fiber types in a pattern consistent across specimens and species (Figure 2). The percentage of slow fibers in M. iliotrochantericus caudalis (-38%) was much higher than the other hip flexors (16-23%).

Knee Flexors

We examined Mm. iliofibularis, flexor cruris lateralis, and flexor cruris medialis, which originate on the synsacrum, insert on the tibiotarsus or fibula, and function as knee flexors. Slow fibers ranged from 2-25% of the total fiber population, in a consistent pattern across all samples of both species (Figure 2).

Ankle Extensors

Three parts of M. gastrocnemius (pars lateralis, pars intermedia, and pars medialis) and plantaris originate from the femur or tibiotarsus, and insert on the tarsometatarsus to extend the ankle. While all

three gastrocnemius parts are very large, plantaris was the smallest muscle examined. The slow fiber distribution was similar for each gastrocnemius part (21-42%) in all individuals, with the exception of one sample of the pars intermedia in one stilt, which had no slow fibers.

Our analysis of available in-flight images and literature revealed a pattern of hindlimb flight posture that was Order-specific. Thus, the Procellariiformes, Podicipediformes, Pelecaniformes, Anseriformes, Phoenicopteriformes, Ciconiiformes, Falconiformes, Galliformes, Gruiformes, Charadriiformes, Gaviiformes, Columbiformes, Psittaciformes, Cuculiformes, Strigiformes, and Caprimulgiformes hold their hindlimbs in the extended posture. The Apodiformes, Trogoniformes, Coraciiformes, Piciformes, and Passeriformes hold their hindlimbs in the flexed posture.





Figure 3 Ring-necked Pheasants, Phasianus colchicus, in take-off (A) and in sustained flight (B). Hindlimbs are in the flexed posture (top arrow) after take-off and are repositioned into the extended posture (bottom arrow) for sustained flight.

The photographs taken at captive facilities revealed that in pheasants and parrots, the limbs were repositioned from a flexed posture following take-off to an extended posture shortly thereafter (Figure 3).

Discussion

All muscles of the hindlimb examined were found to possess both slow and fast fiber types. Each of these muscles, we believe, contributes to establishing or maintaining the extended hindlimb during flight. We expected to find a population of small muscles comparable to those described in American Kestrels that function in maintaining the folded wing (Meyers 1992). Instead, we were surprised to find there is no similar set of muscles functionally dedicated to hindlimb flight posture; rather, the muscles that support the legs are large with mixed fiber types. This may be due to the diverse functions of the hindlimb; the leg is responsible for standing, walking, running, and swimming. These dynamic actions demand a versatile ability, and thus a diverse fiber make-up, of the hindlimb muscles.

Perhaps the most interesting question is *why* there are two hindlimb postures. Shorebirds, raptors, ducks, geese and parrots fly with their hindlimbs extended, whereas perching birds and woodpeckers fly with their legs flexed. In trying to evaluate why two postures exist, we have established a number of hypotheses, outlined below. These five hypotheses offer possible explanation for the diversity of hindlimb flight posture seen in birds. No single hypothesis seems to explain the limb posture for every avian group, nor are they mutually exclusive.

Morphological Hypothesis: In-flight hindlimb posture may be determined by structural constraints of the limb; that is, by joint mechanics, joint musculature, or limb size. For example, birds with long heavy legs may not draw them up into the flexed position for flight because the knee and/ or ankle joints cannot be held in that position. One problem with this hypothesis is that Sandhill Cranes, *Grus canadensis* (Nesbitt 1978, Alonso 1985), Whooping Cranes, *Grus americana*, Canada Geese, *Branta canadensis* (Bard & Lahrman 1965), and Black-crowned Night Herons, *Nycticorax nycitcorax* (Davis 1954), which normally fly with their legs extended, have been observed flying in cold weather with

their hindlimbs in the flexed position. These examples show that the hindlimbs are physically capable of flexion at the knee and ankle and that structural components are not a determining factor for in-flight hindlimb posture.

Ecological/Behavioral Hypothesis: In flight hindlimb posture may be a behavioral component of flight control. Species with relatively short tails (e.g., alcids and procellariformes) use their feet as rudders. Extended hindlimbs can compensate for shortness in the tail or be used to increase the tail width.

Thermodynamic Hypothesis: In-flight hindlimb posture may be related to thermoregulatory needs. Birds may extend or flex their hindlimbs to aid in heat dissipation or conservation. Udvardy (1983) demonstrated that hummingbirds adjust the position of their feet according to ambient air temperatures and subsequent increased body temperature during flight. Regulation of body temperature through the legs has also been demonstrated in pigeons (Martineau & Larochelle 1988). Thus, an extended in-flight hindlimb posture may offset the limited capacity for body temperature cooling through other systems of the body.

Aerodynamic Hypothesis: Hindlimb posture may have an effect on body streamlining during flight. Csicsaky (1977) demonstrated that the shape of the avian body can provide aerodynamic lift. Thus large hindlimbs could disrupt body-lift generation. Therefore, hindlimbs that are small enough to be sufficiently tucked into the body feathers are held in the flexed posture. Large hindlimbs are held in the extended posture in order to prevent disruption in the airflow around the body.

Additionally, hindlimb posture may be modified at different stages of flight. We have observed a change of limb positions, also reported by Barrett-Hamilton (1903), in Ring-necked Pheasants, *Phasianus colchicus*, and in various macaw species. Following take-off, the hindlimbs are drawn up into a flexed posture and may remain there for brief flights. For sustained flight, the legs are quickly repositioned after take-off into the extended posture (Figure 3). We believe this "transitional positioning" is related to the maintenance of necessary aerodynamic forces. We also believe that this is a widespread but little-noted phenomenon that likely contributes to confusion of the hindlimb posture of some birds (see Townsend 1909).

Phylogenetic Hypothesis: The hindlimb posture a bird uses may represent an ancestral trait and may not necessarily be adaptively driven. For example, Ravens, Corvus corax, have relatively large feet but carry them in the flexed posture characteristic of members of their taxonomic order, Passeriformes, and not in the extended posture that might be expected based on their larger hindlimb size. Evaluation of many avian species and their taxonomic orders suggests that bird orders are homogeneous for extended or flexed in-flight hindlimb posture.

We have been unable to determine the flight posture of birds in the order Tinamiformes. Because of their basal position within avian phylogeny, we hope that future examination of tinamous could reveal the ancestral form of flight posture.

Acknowledgments

We would like to thank Dr. John Cavitt for providing specimens, statistical assistance, and commenting on the manuscript. We would also like to thank three anonymous reviewers. Ami Uchida, Karalee Lemmon, and April Adair helped with fiber counts and measurements. Pictures were obtained at the San Diego Zoo and Wild Animal Park (San Diego, CA), the National Aviary (Pittsburgh, PA), and the Diamond Ranch (Syracuse, UT). Skeletal material was provided by the National Museum of Natural History (USNM 555312: Recurvirostra americana). The anti-slow antibody was obtained from the Developmental Studies Hybridoma Bank developed under the auspices of the NICHD and maintained by the Department of Biology, University of Iowa, Iowa City 52242. Supported by a WSU Phyllis Crosby Gardner Undergraduate Research Scholarship (to LSS), an NSF-REU award and a WSU Undergraduate Research Grant (to JCM), an NIH Grant #DC004390 (to RAM), and WSU.

References

Alonso, J. C. (1985). Why Do Cranes Sometimes Fly With Their Legs Drawn Up? J. Field OrnithoL, 56(4), 409-410.

Bard, F. G., & Lahrman, F. W. (1965). Sandhill Cranes Flying with Feet Drawn Up. Blue Jay, 23, 121.

- Barrett-Hamilton, G. E. H. (1903). On the Position Occupied by the Legs of Birds During Flight. *The Zoologist, April*, 139-149.
- Baumel, J. J., King, A. S., Breazile, J. E., Evans, H. E., & Vanden Berge, J. C. (1993). *Handbook of avian anatomy: Nomina Anatomica Avium*, (2nd ed). Cambridge, MA. Nuttall Ornithol Club.
- Csicsaky, M. J. (1977). Body-Gliding in the Zebra Finch. Fortschr. Zool, 24, 275-286.
- Epp, H. T. (1970). Unusual Flight Behavior of Whooping Cranes in Saskatchewan. *Canad. Field-Nat*, 84, 307-308.
- Goldspink, G. (1980). Locomotion and the sliding filament mechanism. In: Aspects of Animal Movement (H.Y. Elder and E.R. Trueman, eds). Cambridge, MA. Cambridge University Press.
- Martineau, L., & Larochelle, J. (1988). The Cooling Power of Pigeon Legs. *J Exp. Biol*, 136, 193-208.
- McFarland, J., & Meyers, R. A. (2005). Anatomy and Histochemistry of Hindlimb Flight Posture in Shorebirds. *Integrat. Comp. Biol*, 45(6), 1166.
- McFarland, J., & Meyers, R. A. (2008). Anatomy and Histochemistry of Hindlimb Flight Posture in Birds. 1. The Extended Hindlimb Posture of Shorebirds. *J. Morphol.* (in press)
- Meyers, R. A. (1992). The Morphological Basis of Folded-Wing Posture in the American Kestrel, *Falco sparverius*. *Anat. Rec*, 232(4), 493-498.
- Meyers, R. A. (1997). Anatomy and Histochemistry of Spread-Wing Posture in Birds. 1. Wing Drying Posture in the Double-Crested Cormorant, *Phalacrocorax auritus*. *J. Morphol*, 233(1), 67-76.
- Meyers, R. A., & Mathias, E. (1997). Anatomy and Histochemistry of Spread-Wing Posture in Birds. Gliding flight in the California Gull, Larus californicus: A paradox of fast fibers and posture. J. Morphol, 233(3), 237-247.
- Meyers, R. A., & Stakebake, E. F. (2005). Anatomy and Histochemistry of Spread-Wing Posture in Birds. 3. Immunohistochemistry of Flight Muscles and the "Shoulder Lock" in Albatrosses. J. Morphol, 263, 12-29.
- Nesbitt, S. A. (1978). Sandhill Cranes in Florida flying with their legs drawn up. *Florida Field Nat*, 6(1), 17-18.
- Rosser, B. W. C., & George, J. C. (1986). Slow Muscle Fibers in the Pectoralis of the Turkey Vulture (*Cathartes aura*): An Adaptation for Soaring Flight. *Zool. Anz*, 217, 252-258.
- Rosser, B. W. C., Waldbillig, D. M., Macdonald, W., & Bandman, E. (1994).

- Muscle Fiber Types in the Pectoralis of the White Pelican, a Soaring Bird. *Acta Zool*, 75, 329-336.
- Shepard, L. S, & Meyers, R. A. (2006). Hold 'Em or Fold 'Em? Hindlimb Flight Posture in Birds. *Integrat. Comp. Biol.* 46(supp 1), e249.
- Torrella J. R., Fouces, V., Palomeque, J., & Viscor, G. (1998). Capillarity and Fibre Types in Locomotory Muscles of Yellow Legged Gulls (Larus cachinnans). Physiol. Zool, 71(4), 425-434.
- Townsend, C. W. (1909). The Position of Birds' Feet in Flight. Auk, 26(2), 109-116.
- Udvardy, M. D. F. (1983). The Role of the Feet in Behavioral Thermoregulation of Hummingbirds. *Condor*, 85, 281-285
- Walkinshaw, L. H. (1953). Notes on the Greater Sandhill Crane (*Grus Canadensis tabida*). Auk, 70, 204-205.

POLITICAL SCIENCE AND PHILOSOPHY

Rejecting Huntington's Global Abridgement: Testing the Clash with an Analysis of Identity in Taiwan

Zachary Vono

Faculty Mentor: Leah Murray

Abstract

The Clash of Civilizations paradigm is one of the most important and influential theories of the post-Cold War era, and applying the Clash's predictive and descriptive claims to East Asia, and Taiwan specifically tests the theory's usefulness. An analysis of identity in Taiwan, drawn from diverse sources, reveals that the Clash paradigm fails to predict or describe behavior in one of the globe's most crucial flashpoints. Taiwan, despite moving closer to mainland China economically, has moved away from it politically.

Introduction

Samuel Huntington's Clash of Civilizations theory, simply put, posits that the world can be divided into eight distinct religious/cultural blocs, and that friction between these "Civilizations" will be the primary cause of conflict in the post-cold-war world. Huntington's prescription to prevent war is to limit inter-civilizational disputes by allowing each civilization to come under the undisputed hegemony of a regional core state (Huntington, p. 316-21). If this is truly the path to peace, it means a massive reordering of American power and an entirely new attitude toward foreign intervention. It is crucial to determine, then, whether or not Huntington is correct. When empirically tested in Asia, however, the Clash theory fails Huntington's own criteria for a successful paradigm: it does not work as a descriptive or predictive tool

If global politics has indeed been increasingly aligning along cultural lines, there should be ample evidence of this from the nearly 15 years since Huntington's theory was born. In search of this evidence, it is most useful to scrutinize East Asia, or what Huntington has termed "Sinic Civilization." This is an ideal region for a test of the Clash thesis: according to Huntington, Sinic is one of the "Challenger Civilizations" which possesses the dynamism to challenge the West, and it contains China, one of the strongest "core states" in the world. In addition, the Sinic Civilization borders on nearly all other Civilizations. Therefore, if the Clash theory is useful elsewhere, it must be invaluable as a predictive and explanatory tool in East Asia.

Huntington's definition of Sinic Civilization is somewhat fuzzy, but it contains at least China as the core state, Hong Kong, Singapore, Taiwan, and the Koreas. It may also include Vietnam and the Chinese Diasporas in Southeast Asia. In addition to ethnicity, Confucian heritage is considered a deciding factor in determining which countries comprise this civilization, although Japan is excluded (Huntington,

Huntington asserts that "a crucial test of a paradigm's validity and usefulness is the extent to which the predictions derived from it turn out to be more accurate than those from alternative paradigms" (p. 37). Therefore, following Huntington's own criteria, for the Civilizational model to be useful it must be successful not only as a descriptive tool, but as a predictive tool as well. Under Huntington's paradigm, nations within Civilizational boundaries should respond similarly given the same stimulus, whether it is cultural, economic, or political. Case studies can show whether or not this is true.

Taiwan: The Other China

Taiwan is overwhelmingly Chinese in ethnicity, boasting a population that is 98% Han Chinese (Dittmer, 2004, p. 476). It has been populated for centuries, but after 1949 the freshly arrived Kuomintang (KMT) ruled the island's population with strict martial law. Chiang Kai-Shek and his successors in the KMT embarked on a campaign to Sinicize the island, suppressing the Taiwanese identity that previously existed in the area (Lynch, 2004, p. 517-519). In the late eighties and early nineties, the government began to democratize, and the ruling KMT Mainlanders were slowly replaced by native Taiwanese politicians. The KMT is still a major political party, but it has moved considerably to the center, no longer resembling Chiang Kai-Shek's Chinesenationalist Kuomintang (Freedom House, Taiwan, 2007).

To Huntington, the 1949 fracture of China is a Cold War relic, and he predicted that China and Taiwan would come together through a common cultural affinity as Germany had. Increasing economic ties were to assist in drawing the island back to its historical position as a province of Imperial China (Huntington, p. 28). Yet, in the past decade Taiwan has undergone a period of intense desinicization, emphasizing its distinct historical and cultural identity. The most recent example of this is the upcoming national referendum on UN membership, which seeks to establish popular support for Taiwan's application for membership into the United Nations. This will be nowhere near the first time the island has shown interest in joining the UN, but recently Taipei has begun using the name "Taiwan" rather than "Republic of China" in its attempts (BBC News, 2/1/08). Even if the referendum passes, as is likely, the UN will likely block the bid due to Chinese pressure. Yet the application under the name Taiwan is a telling barometer of identity. Previously, Taiwan has claimed to be the legitimate government of China. But Taiwan no longer claims to represent all of China, or even part of it. Taiwan is moving away from the mainland in the terms by which it identifies itself on the world stage.

Taiwan has also sought to remove the Sinic legacy of the pre-democracy KMT authoritarians. In May of 2007, Taipei renamed the Chiang Kai-shek Memorial Hall the Taiwan Democracy Memorial Hall (BBC News, 1/1/08). This is far from the only example of this trend. Recently, Taipei has sought to remove Kai-shek from the public consciousness, eliminating the symbol of Taiwanese Sinicization from schools, government facilities, and Taiwan's primary airport.

This push for Taiwanese identity has several possible outcomes. Chief among these are full independence or a continuation of the status quo (Dittmer, p. 477). Full independence is, for the time being, unlikely, due to the imposing military forces the PRC has arrayed against secession. The status quo is widely favored by the Taiwanese people out of pragmatic concern over the threat of invasion by China in the event of independence, and the suppression of democracy in the case of unification.

But Taiwan is being pulled in several directions—toward the mainland by economics and toward its own nationhood by identity. Yun-han Chu, a distinguished Taiwanese scholar, describes the two parallel trends in contemporary Taiwan with regards to the Mainland as 1) increasing economic integration and interdependence, and 2) increasing support for independent national identity (p. 485). In order to determine the dominant trend, we must ask whether identity follows economics, or as Chu puts it, whether "... the transformative power of economic integration has the potential to dissolve (and resolve) the security conflict across the strait at its root, i.e., at the level of the construction of identity, which comes before the formation of national interest and security preference" (p. 496). To answer this question, a closer look at the economic connections between the PRC and ROC is required.

Economic Opportunism or Cultural Affinity?

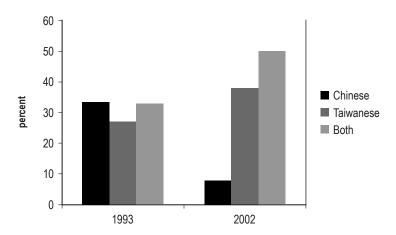
The economic connections formed between Taiwan and the People's Republic of China in the last decade have been staggering. Yet, one must ask whether economic links indicate a cultural affinity and the reorientation of a nation, or whether they simply indicate capitalist opportunism. Huntington himself states: "Economic exchange brings people into contact; it does not bring them into agreement" (p. 218). The proponent of the Clash would likely argue that while economics provides the contact, it is the shared common Chinese culture and history of the two players that will produce agreement. If this is true, we should be seeing a corresponding Taiwanese identification with Mainland China proportionate to the rising economic interdependence.

Yet we see the opposite—the trend towards a unique Taiwanese identity is rising, not falling. In 1993, 33.4% of Taiwanese residents identified themselves as exclusively Chinese. By 2002, this number had plummeted to 7.9%. The number of citizens identifying themselves as exclusively Taiwanese rose from 27.1% to 38% in the same time period. The number of people claiming a "dual identity," that is, both Taiwanese and Chinese, rose from about 33% to around 50% (Chu p.500-1). Therefore, economic integration has not reoriented the

83 • Ergo Featured Articles • 84

identity of those living in Taiwan toward Beijing, and may indeed have had the opposite effect.

Identity in Taiwan 1993-2002



Source: Yun-Han Chu, "Taiwan's National Identity Politics and the Prospects of Cross-Strait Relations," Asian Survey Vol. 44, no. 4 (July/August 2004): 484-512.

There is a significant question as to whether the nationalist trend that has swept Taiwan actually represents the popular identity of the majority of Taiwanese, or whether it is a deliberate attempt at nation building by the pro-independence Democratic Progressive Party (DPP). The DPP and other Taiwanese nationalists have undoubtedly made deliberate desinicization efforts. Pro-independence leaders, such as former president Lee Teng-hui, have cultivated an intellectual campaign to foster a "strong collective identity" among residents of Taiwan (Lynch p. 513-6). Chen Shui-bian, president from 2000-08, is an openly pro-independence Taiwanese nationalist who shepherded a series of political moves designed to instill a Taiwanese identity. He oversaw a resurgence in the Taiwanese language, reduced the presence of China in school curricula, removed Chiang Kai-Shek from the street signs and monuments of the island, and irritated Beijing more than once with his brash rhetoric (Fahey, p. 275-6).

Yet the trend towards a unique Taiwanese identity could not be solely created or sustained by elites; it cannot be without significant

popular origin. After all, the cards are stacked against a Taiwanese identity: it lacks legitimacy in the international community, having been expelled from the UN and rebuffed at its attempts to reenter. In addition, the growing strength of China means that few governments are willing to engage Taiwan on a meaningful political level, and direct military or political assistance from the United States is far from guaranteed. That the DPP has achieved any success in its nation building campaign points to an underlying Taiwanese self-assertion. However, the massive support won by the opposition KMT favoring the status quo and pragmatism in the 2008 elections suggests that many Taiwanese voters would prefer a less antagonistic approach than that adopted by former-president Chen. It is also important to note that even the China-friendly KMT supports Taiwanese membership in the UN (BBC News, 1/12/08).

One final barometer of the importance of Taiwanese nationalism is Beijing's belief that an invasion of Taiwan would entail fighting a determined insurgency. According to the U.S. Department of Defense, this is one of the main deterrents to a PRC invasion of the island. If the Mainland and the island were coming together in any significant cultural or political way, it is unlikely that an insurgency would be a major concern, yet Taiwan has a strong enough national identity to fight a guerrilla war against the PRC that could "tie up [Chinese] forces for years" (U.S. DOD, p. 33-4).

Conclusion

It is evident that the *Clash of Civilizations* model fails both as a predictive and as a descriptive tool in regards to Taiwan, and this raises serious doubts about the theory's effectiveness on the world stage. Huntington's predictive claim that this "other China" would come under the leadership of Beijing has not occurred, nor has it begun to occur. Indeed, despite growing closer economically, the island has moved away from the Mainland politically.

The Clash's descriptive claim, that is, the idea that the common Sinic culture of the Mainland and Taiwan is more important than national or ideological identities, has also proven misleading when not downright false. Despite their close ethnic and economic ties, lumping

the two Chinas together produces a severe misunderstanding of the people on both sides of the Taiwan Strait, and ignores the element of choice in determining national identity. Therefore, anyone attempting to understand this extremely important flashpoint in global politics would do best to avoid the Clash paradigm.

References

- BBC News. (2008). Taiwan recycles Chiang landmark. Retrieved January 1, 2008, from http://news.bbc.co.uk/2/hi/asia-pacific/7167194.stm
- BBC News. (2008). Taiwan set to hold UN referendums. Retrieved February 1, 2008, from http://news.bbc.co.uk/2/hi/asia-pacific/7222531.stm
- BBC News. (2008). Taiwan's opposition in huge win. Retrieved January 12, 2008, from http://news.bbc.co.uk/2/hi/asia-pacific/7184448.stm
- Chu, Yun-Han. (2004). Taiwan's national identity politics and the prospects of cross-strait relations. *Asian Survey*, 44(4), 484-512.
- Freedom House. (2007). Freedom in the World, Country Reports: Taiwan. Retrieved November 4, 2007, from http://www.freedomhouse.org/template.cfm?page=363&year=2007&country=7283
- Huntington, S. P. (2003). *The Clash of Civilizations and the Remaking of World Order*. New York: Simon & Schuster.
- Dittmer, L. (2004). Taiwan and the issue of national identity. *Asian Survey*, 44(4), 475-83.
- Fahey, M. (2007). Taiwanese politics gets interesting again. *Current History*, 107(701), 275-80.
- Lynch, D. C. (2004). Taiwan's self-conscious nation-building project. *Asian Survey*, 44(4), 513-33.
- Mengin, F. State and Identity. (1999). In *Democratization in Taiwan*, ed. Steve Isang and Hung-mao Tien, 116-29. New York: St. Martin's Press.
- U.S. Department of Defense. (2007). *Annual Report to Congress: Military Power of the People's Republic of China*.

SOCIOLOGY AND ANTHROPOLOGY

Did You Teach Them To Say Thank You? Teaching and Evaluating a Social Skills Curriculum

Ronald W. Partridge, Lori Lundell & Shelly Pace*

Faculty Mentor: Brenda Kowalewski

Abstract

This research is part of an ongoing longitudinal study that is evaluating the effect of a youth development program called Youth Impact on a group of youth who attend the program. In this particular phase of the study, the effectiveness of a social skills curriculum called Skillstreaming is the focus. During this phase of the research, this study set out to answer two questions: 1) would the participants who went through the Skillstreaming curriculum significantly improve their social skills from time one to time two?; and 2) would the participants who went through the curriculum have better social skills than those who were not taught the curriculum? Skillstreaming is a curriculum that has been designed to effectively teach different aspects of social interaction such as apologizing, listening, saying thank you, following instructions, and giving compliments. It is structured to teach students using lecture, modeling, role play, course work, and performance feedback. The Skillstreaming curriculum has most often been used in school settings on a specific age group of youth. In this study, the curriculum is taught in a youth development program serving a wide age range of youth who are 9 to 18 years of age. Methods used to collect data include surveys, observation, and analysis of homework assigned in the curriculum. There were a total of 70 participants involved in this study ranging in age from 9 to 18. These participants were assigned to either a control group (N=35) or an experimental group (N=35). Data collection took place at two points in time, before and after completion of the curriculum, via a survey administered to each participant along with their parents and the Youth Impact staff. In addition to the surveys, observation of participants in both the control and experimental groups were recorded between September and November 2006. The expected outcomes of this study are participants who were assigned to the experimental group would significantly improve their skill and frequency in use of the five social skills taught after completion of the Skillstreaming curriculum and participants who have completed

^{*} Weber State University Undergraduate Research Scholarship Travel Award

the curriculum would have substantially better skills than those who did not attend the Skillstreaming curriculum. The results of this study will aid in the overall evaluation of the Youth Impact program.

Introduction

In many youth programs as well as in many schools, instructors are facing an increasing amount of negative aggression from young people as these youth try to deal with both difficult and mundane daily problems (Goldstein, Palumbo, Striepling, & Voutsinas, 1995). Aggressive adolescents are often proficient in antisocial ways of responding to real or imagined provocations but weak in carrying out various prosocially constructive alternative behaviors (Goldstein, Palumbo, Striepling, & Voutinas, 1997). One of the oldest pedagogies in a social skills training curriculum has been skillstreaming. Developed by psychologists, skillstreaming is the process of instructing students using a five step process based on Bandura's social learning theory to teach a variety of social skills.

Bandura's social learning theory states that we learn behaviors by observing others and imitating them (Bandura, 1965). Skillstreaming stresses the importance of positive feedback from peers and trainers, which is a concept Bandura also emphasized, referring to this as self-efficacy. Bandura's theories of modeling and self-efficacy have been utilized and proven successful in numerous research areas such as sports, academics, and the business world (Gould, Hodge, Peterson, & Giannini 1989; Bandura & Wood, 1989; Wood, Bandura, & Bailey, 1990). Skillstreaming has also been influenced by the behaviorist concept of conditioning which originated from John Watson and was refined by such people as Edward Thorndike and B. F. Skinner. Conditioning is the process by which an individual may be shaped and molded through the application of reinforcements and punishments (Skinner, 1953).

Skillstreaming is a curriculum that has been designed to teach different aspects of social interaction, such as apologizing, listening, saying thank you, following instructions, and giving compliments. Several studies on groups found to be deficient in social skills have shown that a skillstreaming curriculum has been effective in changing

behavior, yet admit that further tests of skillstreaming efficiency are necessary (Goldstein, Palumbo, Striepling, & Voutsinas, 1997; Goldstein, Blancero, Carthen, & Glick, 1994; Beeker & Brands, 1986; Cobb, 1973).

The current study specifically analyzes the effectiveness of skill-streaming at a youth development program in the western United States. The objective of this community-based research project is to evaluate the effectiveness of a five week social skills curriculum taught to youth participating in an after-school youth program. Importantly, this study will determine whether this after-school organization continues to use this curriculum in their program. The effectiveness of the skillstreaming curriculum will be determined by answering two specific research questions. The first research question asks: do the ratings of the youth taking the social skills classes significantly differ from the ratings of the youth who did not take the classes? The second asks: do the social skills ratings of the experimental group significantly improve upon completion of the curriculum? The ratings were based on the score given by participants using a Likert scale.

Methods

Subjects for Study

Participants ranged from fourth graders through seniors in high school from a western school district that attend a specific after-school youth program. This district's after-school youth program is a group center designed to provide a safe and fun environment for at-risk youth in the surrounding area. Data were collected from a total of 66 participants. The demographics of the control group and the experimental group were fairly similar. The average age in the experimental group was 12.43 years compared to 11.6 years for the control group. The gender composition of the experimental group was 50% male, 50% female, while the racial/ethnic composition was 26.5% Caucasian, 52.9% Hispanic, and 20.5% other. The gender composition of the control group was 40% male, 60% female, while the racial/ethnic composition was 30% Caucasian, 40% Hispanic, and 30% other. The experimental and control groups were divided according to who turned in their consent forms first

89 • Ergo Featured Articles • 90

Procedure

The researchers administered a survey to each participant's parents and the staff at the after-school program prior to teaching the curriculum and after completion of the curriculum. Those who participated in the curriculum also completed various coursework assignments over the five-week length of the course, given to them by the researchers as outlined as part of the skillstreaming curriculum.

Measurement of concepts

The specific social skills studied were listening, saying "thank you," giving compliments, following instructions, and apologizing. Because the youth participants spend large amounts of time at home and at the after school program, surveys were administered to the parents and staff to evaluate their progress. Using a Likert Scale, survey items corresponded to the five social skills of interest. For example, "Do you apologize when you have done something wrong?" measures the apologizing skill. Response categories ranged from "never" to "always." The responses of parents of the participants as well as the staff at the program were used to determine the effectiveness of the curriculum. Independent t tests were used to compare the mean scores of the participating and non-participating groups. Paired t tests were also used to analyze the change in the experimental group from time 1 to time 2 of the study. The P < .05 alpha level was the adopted criterion to establish statistical significance in this study.

Results

The results of the first research question, "Do the ratings of the youth taking the social skills classes significantly differ from the ratings of the youth who did not take the classes?" are shown in Table 1.

Table 1 includes the mean ratings of the participants as scored by the staff and their parents upon completion of the curriculum. These mean ratings were evaluated using independent t tests. Statistical analysis of the ratings from the parents yielded significance in the skills of "Saying Thank You" and "Apologizing" (t = 2.307, df = 31, P = .028; t = 3.467, df = 31, P = .002). No other significance was found in either the parent or staff ratings. Analysis between the experimental

Table 1

Comparison of Post-tests	Group Type	N	Mean	Sig. (2-tailed)	
Ctoff Chilletrooming Listoning	Experimental	43	4.40	0.070	
Staff Skillstreaming - Listening	Control	22	4.00	0.070	
Ctoff Chilletenessing Coning Theory Van	Experimental	43	4.33	0.046	
Staff Skillstreaming - Saying Thank You	Control	22	4.05	0.246	
Staff Skillatraaming Compliments	Experimental	43	3.88	0.050	
Staff Skillstreaming - Compliments	Control	22	3.36	0.053	
Ctoff Chillatera pairs - Fallaction Instructions	Experimental	43	4.14	0.100	
Staff Skillstreaming - Following Instructions	Control	22	3.68	0.102	
Ctoff Chillatera prairies. A mala simina	Experimental	43	4.09	0.000	
Staff Skillstreaming - Apologizing	Control	22	3.77	0.228	
Derent Chilletreeming Lietaning	Experimental	25	4.12	0.338	
Parent Skillstreaming - Listening	Control	9	3.78	0.550	
Parent Skillstreaming - Saying Thank You	Experimental	25	4.60	0.000	
Parent Skillstreaming - Saying Mank fou	Control	8	3.75	0.028	
Devent Chilleten ensing Compliments	Experimental	25	3.92	0.143	
Parent Skillstreaming - Compliments	Control	8	3.25		
Perent Ckilletragming Following Instructions	Experimental	25	4.00	0.185	
Parent Skillstreaming - Following Instructions	Control	8	3.38		
Parant Skilletraaming Apalogizing	Experimental	25	4.44	0.000	
Parent Skillstreaming - Apologizing	Control	8	3.13	0.002	

group and the control group at time one revealed no significance except within one skill (following instructions) rated by the staff (t = 2.310, df = 47, p = .025).

The results of our second research question, "Do the social skills ratings of the experimental group significantly improve upon completion of the curriculum?" are shown in Table 2.

Table 2 includes pre- and post-test mean ratings of the experimental group as scored by the staff and parents. Paired *t* tests were used to compare the ratings of the experimental group from time one to time two of the study. Statistical analysis of the staff data found statistical significance for each of the five social skills taught: listening, saying

91 • Ergo Featured Articles • 92

"thank you," giving compliments, following instructions, and apologizing respectively (t = -6.338, df = 33, P < .000; t = -5.424, df = 33, P < .000; t = -7.540, df = 33, P < .000; -4.408, df = 33, P < .000; t = -5.447, df = 33, P < .000). Analysis of the parent data found significance in three of the skill areas: saying "thank you," giving compliments and apologizing respectively (t = -3.196, df = 21, P = .004; t = -2.925, df = 21, P = .008; t = -2.318, df = 21, P = .031).

While the impact of consistent attendance was not formally hypothesized, statistical analysis found that attendance did have an effect on the participants in the experimental group. Those participants who attended at least four of the five weeks that made up the cur-

Table 2

1 Staff Skillstreaming - Listening Oct. 2006 3.62 34 0.888 Nov. 2006 4.44** 34 0.746 Staff Skillstreaming - Chair Thank York Oct. 2006 3.82 34 0.834	6 0.128
Listening Nov. 2006 4.44** 34 0.746	* ***
	4 0.143
Saying Thank You Nov. 2006 4.56** 34 0.705	5 0.121
3 Staff Skillstreaming - Oct. 2006 2.85 34 0.784	4 0.134
Compliments Nov. 2006 4.09** 34 0.830	0.142
4 Staff Skillstreaming - Oct. 2006 3.71 34 0.970	0.166
Following Instructions Nov. 2006 4.32** 34 0.843	3 0.145
Staff Skillstreaming - Oct. 2006 3.53 34 1.080	0.185
9 Apologizing Nov. 2006 4.29** 34 0.715	9 0.123
6 Parent Skillstreaming - Oct. 2006 3.77 22 0.922	2 0.197
Listening Nov. 2006 4.14 22 0.990	0.211
Parent Skillstreaming - Oct. 2006 4.05 22 0.653	3 0.139
Saying Thank You Nov. 2006 4.59** 22 0.734	4 0.157
Parent Skillstreaming - Oct. 2006 3.55 22 0.963	3 0.205
8 Compliments Nov. 2006 4.05** 22 1.090	0.232
Parent Skillstreaming - Oct. 2006 3.73 22 0.883	3 0.188
Following Instructions Nov. 2006 4.05 22 1.174	4 0.250
Parent Skillstreaming - Oct. 2006 3.91 22 0.811	0.173
Apologizing Nov. 2006 4.41* 22 0.734	4 0.157

^{*} p < 0.05

riculum had significantly higher skill ratings in each of the five areas: listening (t = 3.719, df = 41, P = .001), saying "thank you" (t = 2.017, df = 41, P = .050), giving compliments (t = 3.269, df = 41, P = .002), following instructions (t = 3.012, df = 41, P = .004), and apologizing (t = 2.523, df = 41, P = .016).

Discussion

The results of this evaluative research study lend support to previous research that indicate the skillstreaming curriculum is an effective course for improving upon the social skills of those who attend (Goldstein, Palumbo, Striepling, & Voutsinas, 1997; Beeker & Brand, 1986; Cobb, 1973; Goldstein, Blancero, Carthen, & Glick, 1994). While the first research question was not entirely supported, the participants in the experimental group did have significantly better social skills ratings than the control group in two of the five areas. The lack of statistical difference in the remaining three skill areas may be a result of a ceiling effect. Both the control and experimental groups were rated near the high end of the measure. Another plausible explanation may be that a ripple effect was experienced within the center. The participants from both groups had daily interaction and aspects of the class may have been shared between groups. There were also skill posters throughout the youth center and this exposure to material from the curriculum could have impacted the control group as well. The second research question was fully supported. The participants from the experimental group showed significant improvements in each of the five skills taught. Some caution is advised in generalizing these findings as the sample size was relatively small in this project and considering all participants were recruited from one youth center.

One of the problems in considering this data is the remarkably short period in which the social skill was measured. As the study progresses, it is critical that the skills are measured on a consistent basis to make sure that the data is not skewed by some unforeseeable event. It would also be advisable to design and construct a new way of measuring one's social skill level. The current measure may not be sensitive enough to measure all aspects of an individual's social skill aptitude.

The results of this study do support Bandura's social learning the-

^{**} p < 0.01

ory, which indicates that we learn social behavior by observing others and imitating them. As supported by Corsaro, this study also lends support to the theory that children, in a passive role, can be shaped and molded by adult reinforcements.

Future studies may want to investigate specifically how attendance impacts learning as this study found it had a significant influence. Longitudinal data would also be desired to investigate the long-term impact of the skillstreaming curriculum. As this research was conducted using participants who attended the same center, a continuation of this research is needed to increase external validity. Future studies may also want to investigate the possible impact that gender and race could have on the skillstreaming curriculum.

References

- Bandura, A. (1965). Influences of models' reinforcement contingencies on the acquisition of imitative responses. *Journal of Personality and Social Psychology*, 1, 589-595.
- Bandura, A., & Wood, R. (1989). Effect of perceived controllability and performance standards on self-regulation of complex decision making. *Journal of Personality and Social Psychology*, 56, 805-814.
- Beeker, M., & Brands, A. (1986). Social skills training in retardates. *Bedragshtherapie*, 19, 3-14.
- Cobb, F. M. (1973). Acquisition and retention of cooperative behavior in young boys through instructions, modeling, and structured learning. Unpublished doctoral dissertation, Syracuse University.
- Goldstein, A. P., Glick, B., Carthan, W., & Blancero, D. (1994). *The prosocial gang.* Thousand Oaks, CA: Sage.
- Goldstein, A. P., Palumbo, J., Striepling, S., & Voutsinas, A. M. (1995). Break it up: A teacher's guide to managing student aggression. Champaign, IL: Research Press.
- Gould, D., Hodge, K., Peterson, K., & Giannini, J. (1989). An exploratory examination of strategies used by elite coaches to enhance self-efficacy in athletes. *Journal of Sport and Exercise Psychology*, 11, 128-140.
- Skinner, B. F. (1953). Some contributions to an experimental analysis of behavior and to psychology as a whole. *American Psychologist*, *8*, 69-78.
- Wood, R., Bandura, A., & Bailey, T. (1990). Mechanisms governing organizational performance in complex decision-making environments. *Organizational Behavior and Human Decision Processes*, 46, 181-201.

BACHELOR OF INTEGRATED STUDIES

Extraction and Analysis of *Trans-*fat in Girl Scout Cookies

Michael Campbell*

Faculty Mentor: Rodney Hansen

Introduction

Trans-fats are a hot topic in the media today. "*Trans*" refers to the arrangement of carbon atoms in the fat molecule. Very few *trans*-fats are found in nature and none of them are from plant sources. Most naturally occurring unsaturated fats are produced by enzymes that produce "*cis*" arrangements (Berg, Tymoczko, & Stryer, 2002, p. 627).

Trans-fats are mostly produced in the food industry by the partial hydrogenation of *cis*-fats. This process hardens the liquid fats making them more suitable for use in baking. The *trans*-fats that are produced are also more stable than their *cis* counterparts; therefore, the *trans* products have a longer shelf life ("Revealing *Trans*," 2003).

The FDA made it mandatory for food manufacturers to put the amount of *trans*-fat a product contains on the food package label as of January 1, 2006 ("Questions About," 2004). This action was taken in response to scientific studies that showed *trans*-fats increase bad LDL cholesterol and decrease protective HDL cholesterol, thus increasing the risk for heart disease (Hwang & Lee, 2005, p. 51).

Because of the FDA action, many food manufactures are modifying their products to remove the *trans*-fat. However, most products that have been modified still contain *trans*-fat. According to the FDA labeling rules, if a product has less than 0.5g of *trans*-fat per serving, it is reported as zero ("Questions About," 2004).

Recently, Girl Scout Cookies changed the production of their cookies to contain "0 grams *trans-*fat." However, since partially hy-

^{*} Phyllis Crosby Gardner Undergraduate Research Scholarship

95 • Ergo Featured Articles • 96

drogenated oils are listed in the ingredients, it is likely the cookies contain measurable amounts of *trans*-fat less than 0.5g per serving. This project used a Soxhlet extractor to isolate the fat in Girl Scout Cookies and FT-IR ATR spectroscopy to quantify the *trans*-fat in the cookies after the product was changed to "*Trans* free."

Methods

Sample Preparation

A Soxhlet extractor was used to extract the fat from each of the eight varieties of Girl Scout Cookies—Little Brownies, Café Cookies, Thin Mints, All Abouts, Do Si Dos, Samoas, Tagalongs, and Trefoils. The fat from 20 to 30 grams of each cookie was extracted using 100mL of hexane for 24 hours. The hexane was later removed by distillation. An internal standard of 0.1g of Trielaidate was added to 0.9g of each sample to avoid the interference from saturated fat that can occur when using Infrared Spectroscopic methods to quantify low levels of *trans-*fat.

Standard Preparation

Pure samples of Trielaidate (*trans*-fat) and trioleate (*cis*-fat) were obtained from Nu-Chek Prep. Using a milligram balance, solutions of 10 percent, 30 percent, 50 percent, and 100 percent were made.

Measurement of Trans-Fats

A Fortier Transform Infrared (FT-IR) Spectrometer equipped with a 45 degree multi bounce ZnSe ATR cell was used to measure the amount of *trans*-fat in the samples. A calibration curve was first created using the standard solutions of 10, 30, 50, and 100 percent *trans*-fat. The peak at 966cm⁻¹ was electronically integrated using OMNIC software. The parameters for the integration were set at 989cm⁻¹ and 944cm⁻¹ with the default tangent. The resulting peak area and the known *trans*-fat concentration of each solution were used to create the calibration curve (see Figure 1).

A spectrum was then obtained from each of the samples of fat from the Girl Scout Cookies. The peak at 966cm⁻¹ was integrated using the same parameters as the standard solutions.

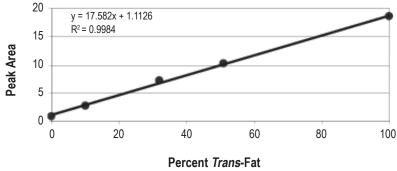


Figure 1 Calibration Curve.

Calculations

The equation of the calibration curve line (see Figure 1) was used to calculate the amount of *trans*-fat in each of the measured samples. The percentage of *trans*-fat added as the internal standard was then subtracted from the total percentage of *trans*-fat in the sample. The resulting percentage represents the amount of *trans*-fat in the sample before the internal standard was added. This percentage was applied to the grams of total fat in one serving of each cookie to determine the number of grams of *trans*-fat in each serving.

Results

All of the cookies contained measurable amounts of *trans*-fat less than 0.5 grams of *trans*-fat per serving. Specific gram amounts of *trans*-fat in one serving of each Girl Scout Cookie are found in Table 1.

 Table 1
 Trans-fat content of Girl Scout Cookies.

Cookie	Grams of Fat per Serving	Grams of <i>Trans</i> -fat per Serving
Trefoils	7g	0.334g
Café Cookies	6g	0.275g
Samoas	8g	0.270g
Tagalongs	10g	0.147g
Little Brownies	8g	0.151g
Do Si Dos	5g	0.140g
Thin Mints	7g	0.136g
All Abouts	6g	0.130g

Discussion

Girl Scout Cookies can legally be labeled zero grams of *trans*-fat. However, they can still be a significant source of dietary *trans*-fat. In 2006 the America Heart Association released new guidelines recommending the consumption of less than 1% of total calories from *trans*-fat, which is approximately 2g per day. Most people will not get more than 1% of their total calories from one serving of Girl Scout Cookies, but every 0.1g adds up, and they will unknowingly increase their risk for heart disease because the label reads zero grams *trans*-fat.

References

- Berg, J. M., Tymoczko, J. L., & Stryer, L. (2002). *Biochemistry* (5th ed.). New York: W. H. Freeman and Company.
- FDA Backgrounder. (2003). FDA acts to provide better information to consumers on trans fats. Retrieved July 4, 2005, from http://www.fda.gov/oc/iniciatives/transfat/backgrounder.html
- Hwang, G., & Lee, D. (2005). *Trans* fat: the latest and worst fat on the block. *Family Practice Recertification*, 27(2), 49-56.
- Questions about *trans* fat nutrition labeling. (2004). Retrieved July 4, 2005, from http://www.cfsan.fda.gov/-dms/qatrans2.html
- Revealing *trans* fats. (2003). *FDA Consumer*, 375, 20-27. Retrieved July 4, 2005, from http://www.cfsan.fda.gov/-dms/fdatrans.html



COMMUNICATION

Cultivation Theory Supports the Effects of Negative News on Society

Mandy J. Seeley

Faculty Mentor: Becky Johns

The recent election results suggest an attitude change in America's voting population. Much has been said about what issues may have spurred the nation to vote out of power Bush-supporting Republicans and into power anti-Bush Democrats. However, little has been said about the media coverage of Bush, the Iraqi War and its effects on the voting. This research and paper demonstrate that the public opinion of President Bush has largely been shaped by what people see in broadcast news and that the high volume of negative news in the past two years directed towards Bush and the war may have swayed Americans' opinions and thus their votes more than any salient issues, information or ideology. Much psychological research demonstrates how negativity can affect people's ideas, feelings and opinions. George Gerbner's research draws a link between violent games and movies with violence. My research utilizes George Gerbner's Cultivation Theory to understand the phenomenon of televised negative news and resulting perceptions about the world in which we live. My intent in this project is to demonstrate that a disproportionately large number of negative news stories affect people's perceptions, resulting in a distorted image of the world. My hope is to educate American citizens about this phenomenon and to encourage those who produce broadcast news to rethink programming content in order to reduce the likelihood of unrealistic negative thinking

This research was presented at the National Conference on Undergraduate Research at Dominican University of California, San Rafael, California, April 12-14, 2007.

CLINICAL LABORATORY SCIENCES

Effects of Hemolysis and Lipemia on Immunoassay Results

Van Aston & Aaron Harper

Faculty Mentor: William Zundel

Hemolysis and lipemia are common interferents in clinical laboratory tests. In high enough quantities they can positively or negatively interfere with testing. Analyte values being measured therefore may be inaccurate. The purpose of this study was to determine what levels of lipemia and hemolysis interfere with specific immunoassays. The 2 categories of assays are competitive and non-competitive (sandwich) assays. The competitive assays include: T3, T4 and testosterone. The non-competitive assays include: TSH, SHBG and IgE. Each analyte was tested with multiple concentrations of interferences in duplicate. All testing was performed on the Roche Diagnostics Modular E170 analyzer at ARUP laboratories in Slat Lake City, Utah. Clinically significant findings are determined at a concentration of interferent that affects recovery of the analyte by +/-10%. Samples from ARUP were pooled together and tested to establish a baseline. Concentrations were prepared from this baseline pool and compared against it. Tests that showed significant effects from hemolysis: IgE, T3 and T4. Tests that showed significant effect from lipemia: SHBG, T3, T4 and testosterone. Some reagent manufacturers do not provide information on common interference with immunoassays. The result of this study encourages individual laboratories to determine acceptable ranges for lipemia and hemolysis interferences.

This research was presented at the American Society for Clinical Laboratory Science Annual Meeting, San Rafael, California, July 17-21, 2007.

CLINICAL LABORATORY SCIENCES

The Effects of the Bordatella Pertussis Toxin on Glycosylated Hemoglobin Levels

Gregory R. Burton, Brett L. Gledhill & Glen R. Trimble

Faculty Mentor: Travis M. Price

Type II Diabetes is one of the most significant medical issues today. Although there are many contributing factors for its development, one that is often overlooked is a decreased sensitivity to the body's natural insulin. Recent studies have shown that a toxin produced by the bacterium Bordatella pertussis has a direct effect on insulin sensitivity, and in turn, glucose levels in animal models. The measurement of glucose and insulin levels are subject to many short term influences and do not provide a clear way to evaluate the effect of this toxin over an extended time period. A test, known as glycosylated hemoglobin or hemoglobin AIC (HgB AIC), is an effective way to monitor the effects of fluctuating glucose and insulin levels over four to six weeks. This study will look at the effects of the Bordatella pertussis toxin on HgB A1C levels in rats. The results of this study will demonstrate the toxin's potential to resensitize an individual who may have become desensitized to insulin. The islet cells of the pancreas play a central role in insulin production and in turn serum glucose levels, so monitoring Hgb AlC will give us an idea of how these cells are responding to toxin stimulation. Our approach will be to establish a normal baseline for glycosylated hemoglobin, performed by high pressure liquid chromatography, in a control and test group. We will inject the test group, subcutaneously in the abdominal region, with the pertussis toxin in solution once a week and obtain a post sensitization sample every four weeks to assess any differences in Hgb AlC and ascertain any existing relationship between these levels and the toxin. The control group will be injected with an equivalent amount of saline solution, subcutaneously in the abdominal region, to simulate the stress caused by toxin injections on the test group. We expect to see lower glycosylated hemoglobin levels in the test group, in response to the toxin, indicating increased insulin sensitivity.

This research was presented at the National Conference on Undergraduate Research at Dominican University of California, San Rafael, California, April 12-14, 2007.

CLINICAL LABORATORY SCIENCES

Comparison of Four Pre-transfusion Methods to Detect Anti-K and Anti-E Antibodies

Chere Clawson, Julie Kakazu, Rodney Sparkman & Elisa Stephenson Faculty Mentor: William Zundel

Transfusion services have a variety of tests to detect clinically significant unexpected antibodies in a patient's serum. These antibodies have the potential to cause hemolytic transfusion reactions (HTR). Transfusion reactions can occur during or shortly following a red blood cell transfusion resulting in compromised patient health or death. Historically, this testing has been done using manual test tube methods in which enhancement media such as polyethylene glycol (PEG) or low ionic strength solution (LISS) is added to a patient's serum to enhance the detection of unexpected antibodies. Recently, semi-automated and automated methods have been introduced to detect these antibodies. Along with this, questions have arisen as to whether these automated methods can meet or exceed the performance of the traditional manual test tube methods. Patient samples with anti-K and anti-E were tested to compare four methods used to detect these antibodies. These methods included two semi-automated methods and two manual tube methods. The antibodies were kept in two separate groups and tested by all four methods using a blind study. It was found that the two semi-automated methods had equal or better sensitivity and specificity than the two manual tube methods in detecting anti-K and anti-E antibodies. Our findings demonstrated that the semiautomated methods have sensitivity and specificity that is greater than or equal to that of PEG. The semi-automated methods and PEG are all greater than the LISS method in both sensitivity and specificity.

This research was presented at the American Society for Clinical Laboratory Science Annual Meeting, San Diego, California, July 17-21, 2007.

CLINICAL LABORATORY SCIENCES

Epidemiology of Community-associated Methicillin-resistant *Staphylococcus Aureas* and Hospital-associated Methicillin-resistant *Staphylococcus Aureas* in Salt Lake City, Utah

Kevin Crandall, Kathy Cox & Vida Schumacher

Faculty Mentor: Travis M. Price

Staphylococcus aureus is one of the most frequently isolated species of bacteria in the clinical laboratory and is implicated in a wide variety of infections ranging from skin infections to pneumonia. Since the advent of antibiotics, some strains of S. aureus have developed resistance to penicillin and more recently methicillin. These resistant strains are known as Methicllin-resistant S. aureus (MRSA).1 The mechanism of methicillin resistance is mediated by a chromosomally incorporated resistance gene referred to as mec A. The mec A gene is sub classified into Staphylococcal Cassette Chromosome mec (SCCmec) types I, II, III, IV, and V.2 Another unique identifier of MRSA is the Panton-Valentine Leukocidin gene (PVL) which has recently been linked to Community Acquired Methicillin-resistant S. aureus (CA-MRSA).3 SCC mec types II and IV as well as PVL gene have been identified in hospital acquired MRSA infections.4 Our goal in this research is to identify the increasing prevalence of community acquired strains of MRSA in hospitalized patients. We will identify the presence of SCCmec types II, IV, and the PVL gene in MRSA strains collected between 2001 and 2005 in the Salt Lake City region. The laboratory analysis will involve Polymerase Chain Reaction (PCR) amplification of the SCCmec cassette types using current hospital procedures. We will be working with a selection from 4,400 bacterial specimens collected from both inpatient and outpatient sources with known MRSA infections from January 1, 2001 to December 31, 2005.

This research was presented at the National Conference on Undergraduate Research at Dominican University of California, San Rafael, California, April 12-14, 2007.

CLINICAL LABORATORY SCIENCES

The Effect and Use of Pilot Tubes on Various Coagulation Studies

Sarah Hansen & Marie Ballif

Faculty Mentor: Yasmin Simonian

The Clinical and Laboratory Standards Institute (CLSI), formerly known as the National Committee for Clinical Laboratory Standards (NCCLS), guidelines state the need for drawing a pilot or discard tube before drawing the 3.2% sodium citrate tube to be used in special coagulation testing. Currently, routine coagulation tests such as Prothrombin Time (PT) and Activated Partial Thromboplastin Time (aPTT) do not require the use of discard tubes. The first tube drawn can be used for testing the PT and the aPTT. When obtaining blood samples for special coagulation tests such as Factor Assays, Antithrombin, Fibrinogen, D-Dimer, and Proteins C and S, the pilot tube is used and the testing is performed on the second tube drawn. To date, PT and aPTT tests are the only tests which do not require a discard tube. The objective of this study is to determine the need for a pilot tube for the following special coagulation procedures. Factor Assays for common factors (VIII, IX and IX), Antithrombin, Fibrinogen, D-Dimer, and Proteins C and S. It is anticipated that the special coagulation testing can be performed on the first tube drawn. The outcome of this study may help eliminate the inconvenience and extra cost of drawing the discard tube. A minimum of thirty healthy volunteers will be recruited and they will have two Greiner Vacuette 3.2% sodium citrate tubes drawn by an experienced and certified phlebotomist. The samples will be spun and aliquoted for testing according to CLSI standards. All tubes will be labeled as tube one and tube two with a unique identification number corresponding with each volunteer to maintain anonymity. Each tube drawn will be tested for Fibrinogen, D-Dimer, Protein C and S, Antithrombin and Factor assays XIII, IX, and XI. Depending on the assay, a 5-10% difference in results between the tubes will be considered significant. A paired t-test will be conducted on results to compare any significant statistical results.

CLINICAL LABORATORY SCIENCES

Comparative Study of Antimicrobial Susceptibility of Uncomplicated Urinary Tract Infections

Michael McQuilkin, Wyatt Palmer & Alex Lund

Faculty Mentor: Travis M. Price

Urinary tract infections (UTI) cause millions of clinical visits every year in the United States with over half of all women reporting at least one UTI in their lifetime. The organisms causing these infections typically respond well to the wide range of antibiotics most commonly prescribed as treatment. Recently, however, there has been an alarming increase in the occurrence of bacterial resistance to these drugs. This resistance causes a number of issues for patients and health care professionals ranging from problems with antibiotic dosage to patient hospitalization. While national trends in antimicrobial susceptibility are tracked and made available to physicians, often the actual patterns of resistance vary by region. This study will look at the antimicrobial susceptibility of organisms isolated from patients with known urinary tract infections in Northern Utah. The isolated bacteria will be subjected to the three most commonly prescribed antibiotics; ciprofloxacin, trimethoprim-sulfamethoxazol, and nitrofurantoin. The aforementioned antimicrobials were chosen specifically due to popularity of prescription and unique characteristics which make accurate medicament vital. This will be done by placing a disc containing a set concentration of each antibiotic onto Mueller-Hinton agar which has been inoculated with the isolated bacteria. After incubation, the bacteria's susceptibility will be measured by the zone of inhibition, or area where the bacteria were unable to grow, around each of the antimicrobial discs. By comparing these zones, we can determine the pattern of sensitivity, for each species of bacteria, to the antibiotics that doctors are most likely to prescribe. This information will allow physicians to more effectively treat urinary tract infections while controlling the growing problem of antimicrobial resistance, an epithet echoed by care givers across the country.

This research was presented at the National Conference on Undergraduate Research at Dominican University of California, San Rafael, California, April 12-14, 2007.

CLINICAL LABORATORY SCIENCES

The Prevalence of Human Metapneumovirus in Pediatric Patients of Utah

Clinton Potter & Devin Christensen

Faculty Mentor: Scott Wright

In the pediatric population, Respiratory Syncitial Virus (RSV), parainfluenza viruses, and influenza viruses account for a majority of the cases of respiratory viral infections that lead to bronchitis and pneumonia. Still, in about 15-34% of respiratory cases the causative agent has remained unknown. A new virus, the Human Metapneumovirus (hMPV), was discovered in 2001. With the advancement in viral testing, it has been proven that 5-10% of patients with respiratory symptoms are infected with hMPV. The objectives of this research study are to demonstrate the prevalence of hMPV in pediatric patients with respiratory infections and determine if it acts as a solitary infectious agent, a co-infectious agent, or both. The researchers expect to find data to implicate both. The samples used in this study will be obtained from patients at Primary Children's Medical Center (PCMC), in Salt Lake City, Utah. Each sample will be analyzed for the presence of hMPV by extracting RNA and making multiple copies of it through a process called RT-PCR (Reverse Transcriptase-Polymerase Chain Reaction). Following testing, previously obtained respiratory panel results will be compared with the RT-PCR results. Overall, this study aims to advance clinical research involving hMPV, thereby aiding in the healthcare of pediatric patients.

This research was presented at the American Society for Clinical Laboratory Science Annual Meeting, San Diego, California, July 17-21, 2007.

Research Abstracts ◆ 110

CLINICAL LABORATORY SCIENCES

Comparison of FDA-Approved Antimicrobials to Non-prescription Mexican Antimicrobials

Trina Riley, Leah Daily & Tyler Roe

Faculty Mentor: Scott Wright

It has become common practice for Americans to cross the border into Mexico to obtain prescription medications without a prescription. This practice is potentially dangerous because the drugs being purchased across the border are not regulated. There is no guarantee that what the consumer is purchasing is what they think it is. The aim of this research is to determine the bacteriacidal level of Mexican antimicrobials as compared to their FDA regulated counterparts. Two commonly prescribed antimicrobials, amoxicillin and ciprofloxacin, will be obtained from at least ten different sources, to include farmacias in Mexico and several online Mexican pharmacies. Using the minimum bacteriacidal concentration (MBC) method, the potency of the Mexican antimicrobials will be compared to FDA regulated drugs from the U.S., obtained via a research prescription from Weber State University's Health Clinic. These antimicrobials will be tested against two common pathogens, Escherichia coli and Staphylococcus aureus; organisms that are often implicated in skin and urinary tract infections. This research aims to determine if the antimicrobial effects of the drugs in vitro are essentially the same compared to U.S. drugs, or are the Mexican antimicrobials more effective or less effective at inhibiting bacterial growth.

This research was presented at the National Conference on Undergraduate Research at Dominican University of California, San Rafael, California, April 12-14, 2007.

CLINICAL LABORATORY SCIENCES

Effect of Stress on Platelet Function

Alisha Weiss, Nathan Fenn & Majak John

Faculty Mentor: Kara Hansen-Suchy

Heart disease remains one of the leading causes of death in men and women throughout the United States. Diet, lack of exercise and genetics have all been proven factors in increasing heart disease risk; however, in the past few years, evidence shows that stress may be a fourth factor in increasing this risk. In this study we will compare the effect of temporary stress stimulation on the effect of platelet function. Subjects will be fifty volunteers from the allied health classes, composed of college aged students (18-30 years), presumably less affected by atherosclerosis, high blood pressure, heart disease, and other disorders that predispose platelet interaction. Certain drugs such as aspirin effect platelet function, therefore our volunteers must be pre-screened for use and those volunteers removed from the study if on those medications. To begin, subjects will have a blood sample and vital signs taken, and complete questionnaires. These will be used as baseline samples. A modification of the Trier Social Stress Test (TSST) will then be used in order to induce stress in the subjects. The modified TSST will be fifteen minutes in length and is composed of three tests assessing mock student performance. We will again take all participants' vital signs and draw blood immediately after the TSST as well as question the perceived level of stress. Some blood work will be performed on a Coulter $Max-M^{TM}$ on campus within four hours of collection, while the platelet function studies will be performed on a PFA-100™, located at a community medical center, also within four hours of collection. Data will be collated and analyzed using Statistical Package for Social Science (SPSS). Our goal in performing this research is to determine if there will be a detectable increase in platelet activity after a stress stimulation session as compared to baseline levels.

GEOSCIENCES

Investigations into the Morphology and Spatial Distribution of Hoodoos at Goblin Valley State Park, Emery County, Utah

Summer L. Day & Steven A. Fellows

Faculty Mentor: Michael Hernandez & Richard L. Ford

The primary attraction at Goblin Valley State Park (Emery County, UT) is the array of interestingly shaped and excellently exposed hoodoos, locally called goblins, formed within the Jurassic Entrada Sandstone. The typical goblin consists of a spherical or oblong cap of resistant sandstone supported by a pedestal of softer siltstone and shale. Previous investigations have demonstrated the importance of spheroidal weathering, concentrated along near-vertical joint sets, in the development of the goblins. The ultimate fate of a goblin is for the sandstone cap to topple from its pedestal, a process which may be entirely natural or significantly accelerated by human activity. The primary goal of this study is to quantify spatial trends in goblin morphology and in the ratio of intact vs toppled goblins, in order to assess the potential human impact. Field investigations during the fall of 2006 focused on an area of goblins rarely accessed by park visitors, located approximately 1.4 km south of the visitor parking area. Here basic morphomteric and GPS data were collected on goblins that lie along two nearly perpendicular transects. In addition, a study transect and preliminary GPS inventory of toppled goblins were started in the area most commonly accessed by park visitors (less than 0.5 km from the parking area). Data collection will continue in spring 2007. Preliminary results indicate an important relationship between a local drainage divide and the overall occurrence of goblins. The west-facing cliff of Entrada Sandstone from which the goblins emerge coincides with the drainage divide between Goblin Valley and Well Draw. Our preliminary transect data indicate a substantial difference in the percentage of intact goblins in the two areas. The two transects in the more distant area have an average of 15 goblin features per 100 m of transect, with over 50% of those features being intact goblins; the remainder are toppled goblins or remnant pedestals. The transect nearest the parking area has an average of 5 goblin features per 100 m of transect, with only 27% of those feature being intact goblins. Future studies are planned to further investigate this trend in hopes of determining if the difference is the result of natural variation in goblin density or the result of human disturbance.

This research was presented at the Geological Society of America Rocky Mountain Session 59th Annual Meeting, St. George, Utah, May 7-9, 2007.

GEOSCIENCES

Petrolology of the Cretaceous Straight Cliffs-Wahweap Formations Transition, Southern Utah

Richard L. Emerson, Joseph R. Goodin & Cameron R. Thompson Faculty Mentor: Jeff Eaton

Five stratigraphic sections were measured and 68 petrographic samples were collected in the upper part of the Straight Cliffs Formation (John Henry and Drip Tank members) and the lower part of the overlying Wahweap Formation on the margins of the Paunsaugunt Plateau including Bryce Canyon National Park (BCNP). These sections and petrographic samples were then compared to samples taken from two measured sections near the type section of the Drip Tank Member of the Straight Cliffs Formation on the Kaiparowits Plateau. Petrographic results show that the lithics in the Drip Tank Member are dominated by chert (>95%) and that the Wahweap lithics are dominated by carbonates (>85%). The chert to carbonate ratio observed within the top 5 meters of the Drip Tank varies widely, consistent with previous work indicating a change in provenance from the Mongollon Highlands to the Sevier orogenic belt marked by the mixing of sediment types. These observations were then applied to a section located approximately 15 km west of BCNP in Hillsdale Canyon along the western margin of the Paunsaugunt Plateau. This section consists of a series of conglomerates rather than the single cliff forming conglomerate that marks the top of the Drip Tank Member elsewhere and as such the boundary between the Straight Cliffs and Wahweap formations in this area has been problematic. This study suggests that petrography can be used to distinguish the Wahweap conglomerates from and Straight Cliffs conglomerates based on the lithic content. The Drip Tank Member of the Straight Cliffs Formation in Hillsdale Canyon can be tentatively assigned a thickness of 74 m, consistent with the 40-132 m thicknesses observed on the eastern margins of the plateau.

This research was presented at the Geological Society of America Rocky Mountain Session 59th Annual Meeting, Saint George, Utah, May 7-9, 2007.

PHYSICS

Mapping of GeSbTe Thin Film Electrical Properties with AFM

Jordan Brocious

Faculty Mentor: Colin Inglefield

Thin films of GeSbTe are of interest due to their potential use in rewritable optical data storage media and reconfigurable electronics. The amorphous and crystalline phases of GeSbTe exhibit very different reflectivity and electrical conductivity. Films of nominally amorphous Ge_2Sb_2Te_5 were grown to various thicknesses using RF sputtering on quartz substrates. The surfaces of the films were analyzed using Atomic Force Microscopy (AFM) and surface roughness measurements were taken. The thicker films had a truly isotropic surface while the thinnest films displayed crystalline features, such as angular steps. Conductivity measurements of the films in both coplanar and sandwich geometries correlate with the AFM data and indicate a high degree of crystallinity during the initial stages of growth. This work was supported by the Air Force Research Laboratory under grant number F29601-03-01-0229 and by Weber State University through the Phyllis Crosby Gardner fellowship.

This research was presented at the American Physical Society Four Corners Section Fall Meeting, Flagstaff, Arizona, October 19-20, 2007.

Zoology

Effect of Mercury on the Brine Shrimp Artemia's Survival

Austin Finklea

Faculty Mentor: Nicole Okazaki

The Great Salt Lake is a terminal lake, receiving influx of fresh water from several rivers and effluents from nearby industries. Recent measurements have shown increasing amount of various salts, including mercury. Since the toxicity of this compound is well documented, we were interested in its effect in the shrimp and in its accumulation along the food chain. Materials and Methods- In a first part, we measured the mortality rates of larvae and adults exposed at various mercury concentrations. In the second part, we raised algae on sea water (SW) at 30 parts per thousand, containing mercury for at least 7 days. We centrifuged the algae to eliminate the SW and re-suspended the cultures with fresh, clean SW. Results- Exposures to low levels of mercury (10-4 g/l), at temperatures of 23 to 25°C, had no significant effect on Artemia larval survival. However, 40% died after 24 hour of mercury exposure at 10-3 g/l. Mortality of 90% and above occurred at concentrations of 10-2 g/l. Interestingly, exposures to mercury at 20°C were 10 fold less toxic than exposure at 25°C. While a trend of higher mortality was found with shrimp larvae fed algae contaminated with mercury (7% in control, 4% in mercury contaminated algae), this trend was not significant. Discussion and conclusion- The higher mortality rate occurring at higher temperatures suggests a variable seasonal response of the shrimp to occasional mercury exposures. Measurements of mercury internalized by the algae and absorbed by the shrimp should be the next step in this study and should enable us to compare these laboratory levels to the ones actually found in the GSL shrimp.

This research was presented at the National Conference on Undergraduate Research at Dominican University of California, San Rafael, California, April 12-14, 2007.

Zoology

The Structure and Function and Temporal Expression of Fibrillin in Zebrafish (*Danio rerio*)

Christian Francom, Joshua McBride & Jeff Caldwell

Faculty Mentor: Barbara C. Trask

Although the sequence of fibrillin is highly conserved among numerous species, the structure and function of this elastic extracellular matrix protein in zebrafish has yet to be described. To better characterize the fish fibrillin protein, we first confirmed the structural similarity of fish microfibrils with their mammalian counterparts in both cillary zonules and the ventral aorta using transmission electron microscopy. Using a published zebrafish fibrillin cDNA sequence (GenBank Accession # XM_43257), oligonucleotide primers were designed to amplify various regions of fish fibrillin that, based upon alignment with the mammalian gene, are likely to encode for putative TGF- β binding-like domains. While the alignment suggests some interspecies conservation, only one of the three amplified regions conserves the consensus cysteine placement suggested to be critical for growth factor binding. These amplified regions have been cloned for expression so they recombinant protein may be used in future functional studies.

This research was presented at the Gordon Research Conference on Elastin and Elastic Fibers at the University of New England, Biddeford, Maine, July 29-August 3, 2007.

Zoology

Developmental Expression of the Microfibrillar Proteins Fibrillin and MAGP in Zebrafish (*Danio Rerio*)

Joshua McBride, Christian Francom & Jeff Caldwell

Faculty Mentor: Barbara C. Trask

Transmission electron microscopy of different tissues suggests that the elastic matrix microfibrils in adult zebrafish have structural similarity with their mammalian counterparts. These structures are known to be comprised of numerous proteins, including fibrillin and microfibril-associated glycoprotein (MAGP), that must be secreted in a temporally- and spatially-regulated manner to give rise to functional microfibrils. While adult zebrafish appear to have functional microfibrils implying proper developmental secretion of their component proteins, the temporal and spatial expression of these proteins during fish development is unknown. To investigate this, we used a published zebrafish fibrillin cDNA sequence (GenBank Accession # XN 43257) to generate oligonucleotide primers that amplify a 678 nt region of fibrillin using RT-PCR. Similarly, using a published Xenopus MAGP-1 cDNA sequence (GenBank Accession # BC041238), primers were designed to amplify a 300 nt portion of the zebrafish MAGP gene. Using whole embryo total RNA isolated at different developmental stages, intensities of the amplified products were normalized to either zebrafish β-actin or glyceraldehyde-3-phosphate dehydrogenase (GAPDH) to determine the timing of peak expression for both genes. In situ hybridization using these PCR products as probes will enable spatial localization of the gene expression for these two microfibrillar proteins at these peak times.

This research was presented at the Gordon Research Conference on Elastin and Elastic Fibers at the University of New England, Biddeford, Maine, July 29-August 3, 2007.

Zoology

Genetic Variation in Natural Populations of the Great Salt Lake Brine Fly, *E. Cinerea*

Brian Oney

Faculty Mentor: Jonathan Clark

The Great Salt Lake (GSL) Ecosystem represents one of the largest bird refuges in North America and includes the largest lake west of the Mississippi river, with a total surface area of 4400 square kilometers. An important component of this harsh and beautiful lake are several species of brine flies (Family Ephydridae). It is estimated that the brine flies annually remove 90 million kg of organic matter from the benthos of the GSL. In addition, they are a principle food source for millions of birds associated with this ecosystem. This study utilized DNA amplification and sequencing techniques to examine genetic variability within the internal transcribed spacer-1 (ITS-1) region of the brine fly, Ephydra cinerea. Because it is a non-coding DNA sequence, the ITS-1 region is highly variable and provides a useful measure for intra-species genetic comparisons. The hypothesis to be tested is that the extent of ITS-1 sequence variation among flies isolated from geographically separated populations exceeds that of flies from the same population. DNA was isolated from flies collected from four regions of the GSL, and the ITS-1 regions were amplified and sequenced. From sequence comparisons, the extent of genetic similarity among individuals is examined by comparing the percent nucleotide identity. This information will be eventually used to examine the extent to which flies from different populations interbreed.

Zoology

Incubation Rhythm and Behavior of Long-billed Curlew at Great Salt Lake

Kyle Stone

Faculty Mentor: John F. Cavitt

The Long-billed Curlew is the largest of all North American shorebirds and is known to breed throughout the Great Plains and Great Basin. Unfortunately, little information is known about the incubation rhythm, duration of attentive periods and changeover activities of this species. We examined incubation behavior by placing miniature video cameras connected to recorders that allow for continuous recording 24 hours per day. The results of these observations suggest that eggs are rarely left unattended and typically just during changeover periods. The male incubated continuously at night and the female throughout the day. During the day, the incubation bout length was variable but averaged 10 hours in duration.

This research was presented at the One Hundred and Twenty-fifth Stated Meeting of the American Ornithologists' Union at the University of Wyoming, Laramie, Wyoming, August 9-11, 2007.

HISTORY

Photographing Folklore: Ogden's Underworld

Beau James Burgess

Faculty Mentor: Kathryn MacKay

"Two-Bit Street," Ogden, Utah is not only a collection of buildings, but a space, a view, an atmosphere, a persona—a cultural landscape. Throughout the history of Ogden, from early railroad days, through the roaring 20's of jazz and drinking, and the war years of troop trains, 25th street has been a crossroads. It was nearly completely demolished, is now being revitalized—a place both transcending time and reflecting it. Urban legends about the street continue to flourish. Stories about gambling, bootlegging, prostitution, and murder—all interconnected to rumors about underground tunnels and lingering ghosts. This project has been about researching folklore archives and newspaper records, collecting additional stories, and photographing sites. I have uncovered "lost" images, documented the "lack" of evidence, and re-photographed historic locations. In the process I have become interested in the interactions between oral, visual, and artifactual ways of knowing.

PSYCHOLOGY

The Effects of Timed Exposure to Light Therapy on Melatonin

Kristena Kons

Faculty Mentor: Lauren A. Fowler

Shiftwork is essential for military personnel working in a 24/7 environment. Many accidents that occur during shiftwork can be pinpointed to fatigued workers with desynchronized circadian rhythms. Fatigue-related accidents cost the U.S. Air Force \$54 million annually, and given the nature of military personnel, these errors also threaten homeland security. Researchers have found that exposure to bright light resynchronizes circadian melatonin rhythms, reducing the amount of fatigue during shiftwork. This study examined whether timed exposure to light therapy would act as a fatigue countermeasure, reducing cognitive and physiological fatigue. Participants included 13 military air traffic and weapon controllers working rapidly rotating shiftwork schedules. Salivary melatonin samples and a computerized cognitive task developed by the military (SynWin) were used to measure physiological and cognitive fatigue. Fatigue measures were taken before and after the administration of light treatment once at the beginning of the morning/day shifts and once at the end of the swing shift. Melatonin and SynWin data were analyzed with a 2 x 2 (shift x light therapy) repeated measures MANOVA. Difference scores were used to condense the means of the pre-test/post-test data. The administration of light treatment resulted in an increased performance in SynWin scores for participants working the morning/day shifts. Light administration significantly suppressed melatonin levels during the swing shift, phase delaying melatonin circadian rhythms. The present findings demonstrate the feasibility of using an inexpensive and portable light therapy device during swing and night shifts in military settings to decrease fatigue related errors among military personnel.

This research was presented at the Council on Undergraduate Research Posters on the Hill, Washington D.C., April 23-27, 2007.

Psychology

The Influence of Light Therapy on Percieved and Cognitive Fatigue During Rapidly Rotating Shiftwork Schedules

Kristena Kons

Faculty Mentor: Lauren A. Fowler

Occupational human errors are often the result of fatigued shift workers with desynchronized rhythms. Researchers have found that exposure to bright light resynchronizes circadian melatonin rhythms, reducing the amount of fatigue during shiftwork. This study was designed to assess the immediate effects of exposure to light therapy on reducing perceived and cognitive fatigue. Thirteen military air traffic and weapon controllers working rapidly rotating shiftwork schedules served as participants. The Stanford Sleepiness Scale (SSS) and a computerized cognitive task developed by the military (SynWin) were used to measure perceived and cognitive fatigue. Data were collected once at the beginning of the morning/day shifts and once at the end of the swing shift sequentially before and after the administration of the light treatment. The SSS and SynWin data were analyzed with a 2 x 2 (shift x light therapy) repeated measures MANOVA. Difference scores were used to condense the means of the pretest/post-test data. The administration of light treatment resulted in an increased performance in SynWin scores for participants working the morning/day shifts, but no significant effect was found in the swing shift. Participants who had the light treatment reported having lower subjective sleepiness in the swing shift than in the morning/day shifts. The present findings demonstrate the feasibility of using an inexpensive and portable light therapy device to decrease fatigue related errors for personnel working rapidly rotating shift work schedules.

PSYCHOLOGY

Time of Day Effects on Mental Stress-related Sympathetic Nervous System Activation in Shift Workers

Caleb D. Wilson

Faculty Mentor: Lauren A. Fowler

Fatigue and stress are the most common complaints of shift workers. They are also the largest contributors to the increase in errors that occurs during shifts outside of normal daytime hours. Stress in shift work has largely been attributed to increased fatigue from working outside of the body's optimal circadian rhythms for performance. This study sought to establish a link between higher stress levels and an actual difference in time of day sympathetic nervous system activation. To examine this link, differences were investigated in the body's physiological stress responses to performing mental tasks during day and evening shifts in military shift workers. United States Air Force air traffic control personnel that switch rapidly between a day shift and a swing shift participated in the study. Each participant was tested during each of their shifts by performing a variation of Stroop's Naming Colored Words Task and a visual matching task from Woodcock Johnson's Cognitive Battery. These tasks were expected to be stress-inducing as they required quick and accurate scanning and filtering of information. Continuous data for heart rate and galvanic skin response were gathered during each testing period. These data were analyzed as indicators of sympathetic nervous system arousal. Heart rate data showed higher maximum values at night than during the day. Galvanic skin response data displayed the same trend of higher maximum values at night. While there were physiological differences between shifts, cognitive performance showed no significant difference between day and swing shifts. The higher maximum values of stress indicators at night show that completion of the tasks during the swing shift resulted in higher stress levels. The major implication of this finding is that while task performance did not differ, stress from task performance was enhanced at night. These differences in sympathetic stress levels may help account for greater fatigue during night and swing shifts.

This research was presented at the National Conference on Undergraduate Research at Dominican University of California, San Rafael, California, April 12-14, 2007.

SOCIOLOGY AND ANTHROPOLOGY

"It's a Crappy Routine": An Evaluation of Shift Workers' Time Management According to Marital Status and Gender

Lori Lundell

Faculty Mentor: Autumn Behringer

While some sociological research has explored the ways in which gender impacts the amount of time men and women devote to paid and unpaid work, a broader analysis of the relationship between gender and time has yet to be conducted. This study investigates how gender intersects with work to produce differences in both the conceptualization of time as well as time prioritization. Due to the unique time pressures and constraints experienced by shift workers we focused our study on this previously under explored group within the population. We recruited thirty shift workers for semi structured interviews that explored the way they thought about and used time. This study creates a framework of analysis that will be useful for future, more systematic studies, while exploring the qualitative differences in how informants conceptualize their time management while participating in shift work. Interview transcripts were coded to analyze the similarities and differences in how informants thought about their private and professional lives. Analysis reveals that gender interacts with martial status to produce significant differences in time management. In addition to these findings, the data also suggest gender differences are more prevalent for married than unmarried individuals.

SOCIOLOGY AND ANTHROPOLOGY

Teaching and Evaluating a Social Skills Curriculum

Ronald W. Partridge, Lori Lundell, Elly Alvarado & Kari Yurth

Faculty Mentor: Brenda Kowalewski

This research is part of an ongoing longitudinal study that is evaluating the effect of a youth development program called Youth Impact on a group of youth who attend the program. In this particular phase of the study, the effectiveness of a social skills curriculum called Skillstreaming is the focus. During this phase of the research this study set out to answer two questions: 1) would the participants who went through the Skillstreaming curriculum significantly improve their social skills from time one to time two?; and 2) would the participants who went through the curriculum have better social skills than those who were not taught the curriculum? Skillstreaming is a curriculum that teaches different aspects of social interaction such as; apologizing, listening, saying thank you, following instructions, and giving compliments. It is structured to teach students using lecture, modeling, role play, course work, and performance feedback. In this study, the curriculum is taught in a youth development program serving a wide age range of youth who are 9 to 18 years of age. Methods used to collect data include: surveys, observation, and analysis of homework assigned in the curriculum. There were a total of 70 participants involved in this study ranging in age from 9 to 18. These participants were assigned to either a control group (N=35) or an experimental group (N=35). Data collection took place at two points in time, before and after completion of the curriculum, via a survey administered to each participant along with their parents and the Youth Impact staff. In addition to the surveys, observation of participants in both the control and experimental groups were recorded between September and November 2006. The expected outcomes of this study are participants who were assigned to the experimental group would significantly improve their skill and frequency in use of the five social skills taught after completion of the Skillstreaming curriculum and participants who have completed the curriculum would have substantially better skills than those who did not attend the Skillstreaming curriculum.

This research was presented at the National Conference on Undergraduate Research at Dominican University of California, San Rafael, California, April 12-14, 2007.

Women's Studies

Un-quieting Women's Voices in the General Membership of the LDS Church

Chelsea Whitby

Faculty Mentor: Becky Johns, Chloe D. Merrill & Sylvia Newman

Many LDS women unnecessarily place self-imposed restrictions on their roles, rights, and responsibilities by inferring limitations on their behavior that do not actually appear in Church doctrine or policy. Men and women have difference roles and responsibilities within the Church but equal responsibility to gain the skills and knowledge necessary for full participation in public Church life. Through authoritative self-definition and expression, increased recognition, respect for the diversity of women's experience, and validation of female independence, the oversimplified conceptions of "women's roles" in the Church could be broadened to be much more substantial, and women's doctrinal reliance could be focused more inwardly.

This research was presented at the Salt Lake Sunstone Symposium in Salt Lake City, Utah, August 8-11, 2007.