

**HANDBOOK**  
**AND**  
**PROCEEDINGS**

OF THE  
**TENNESSEE JUNIOR**  
**ACADEMY OF SCIENCE**

**2010**



Sponsored by the

Tennessee Academy of Science

Edited and Prepared by Jack Rhoton, Director

Tennessee Junior Academy of Science

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TENNESSEE JUNIOR ACADEMY

OF SCIENCE

ANNUAL MEETING

Belmont University

Nashville, Tennessee

April 16, 2010

Sponsored by the

TENNESSEE ACADEMY OF SCIENCE

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TENNESSEE ACADEMY OF SCIENCE OFFICERS: 2010

Michael A. Gibson..... President  
University of Tennessee at Martin, Martin

Jeffery Boles.....President-Elect  
Tennessee Technological University, Cookeville

M. Gore Ervin .....Immediate Past President  
Middle Tennessee State University, Murfreesboro

Teresa Fulcher.....Secretary  
Pellissippi State Technical Community College, Knoxville

C. Stephen Murphree.....Treasurer  
Belmont University, Nashville

Steven J. Stedman.....Editor, *Tennessee Academy of Science Journal*  
Tennessee Technological University, Cookeville

TENNESSEE JUNIOR ACADEMY OF SCIENCE  
Sponsored by the  
TENNESSEE ACADEMY OF SCIENCE

Jack Rhoton.....Director, Tennessee Junior Academy of Science  
East Tennessee State University, Johnson City

READING COMMITTEE 2009-2010

Jack Rhoton.....East Tennessee State University

William N. Pafford.....East Tennessee State University

Gary Henson.....East Tennessee State University

Timothy McDowell.....East Tennessee State University

JUDGES

M. Gore Ervin.....Middle Tennessee State University

Preston J. MacDougall.....Middle Tennessee State University

Elbert Myles.....Tennessee State University

Chih-Che Tai..... East Tennessee State University

LOCAL ARRANGEMENTS

Dr. Steven Murphree.....Belmont University

## INSTRUCTIONS FOR PARTICIPATION IN THE TENNESSEE JUNIOR ACADEMY OF SCIENCE

**Purpose.** The Tennessee Junior Academy of Science (TJAS) is designed to further the cause of science education in Tennessee high schools by providing an annual program of scientific atmosphere and stimulation for capable students. It is comparable to scientific meetings of adult scientists. The Junior Academy supplements other efforts in the encouragement of able students of science by providing one venue of stimulation and expression

**Rewards and Prizes.** The student's primary rewards are the honor of being selected to appear on the program, experience in presenting his/her paper, opportunity to discuss this work with other students of similar interests, membership in the Tennessee Junior Academy of Science, and publication of his/her paper in the *Handbook and Proceedings of the Tennessee Junior Academy of Science*. However, the top two student writers will receive \$500 each from the Tennessee Academy of Science, and other top writers will receive \$200 for each paper published in the *Handbook*. In addition, the TAS will award \$500 to each of the top two writers to participate in the Annual Meeting of the American Junior Academy of Science (AJAS). The AJAS meeting is held in a different city each year. All students who present papers to the TJAS are encouraged to enter their papers in other competitive programs, such as the Westinghouse Science Talent Search and the International Science and Engineering Fair. Students are also encouraged to solicit scholarships from individuals, companies, or institutions.

**Preparation of the Report.** The report should be an accurate presentation of a science or mathematics project completed by the student. It should be comprehensive, yet avoid excessive verbosity. Maximum length should be 2000 words. The report and the project it describes must be original with the student, not just a review of another article. It should be obvious that the experimentation and/or observations have been scientifically made. The paper should reflect credit on the writer and the school represented.

Visual aids such as slides, mock-ups, and charts may be used in presentation of the report. **PLEASE NOTE THE FOLLOWING: ILLUSTRATIONS WITHIN THE REPORT MUST BE RESTRICTED TO TABLES AND/OR SIMPLE LINE DRAWINGS. These must be done in BLACK ON 8 ½ X 11 WHITE PAPER. COLORED FIGURES CANNOT BE PRINTED IN THE HANDBOOK. Total width of the illustration itself cannot be more than 7". Illustrations submitted with the paper MUST be originals, NOT COPIES, and MUST be BLACK AND WHITE.**

The report must be **DOUBLE-SPACED** on 8 ½" by 11" paper. Give careful attention to spelling and grammar. **IT IS VERY IMPORTANT that YOU prepare a COVER SHEET** for the report, giving **ALL** the required information as specified, **INCLUDING YOUR HOME TELEPHONE NUMBER AND E-MAIL ADDRESS**. IF YOUR PAPER SHOULD BE SELECTED FOR PUBLICATION, IT MAY BE NECESSARY FOR OUR EDITORS TO CONTACT YOU. FAILURE TO PROVIDE CONTACT INFORMATION COULD PREVENT YOUR PAPER FROM BEING PUBLISHED. The

cover sheet included with this material may be duplicated as needed. Prepare an abstract to accompany your paper (not more than 100 words). **NO PAPER WILL BE CONSIDERED UNLESS IT IS ACCOMPANIED BY AN ABSTRACT.**

**Scientific or Technical Report Writing.** A very important phase of the research of a scientist is the effective reporting of the research project attempted and completed. The technical report is different from other kinds of informative writing in that it has a single, predetermined purpose: to investigate an assigned subject for particular reasons. Technical reporting is done in the passive voice. Use of personal pronouns should be avoided except in rare instances. The telling portion of the research job is often underrated. Thus, communication is a very necessary part of research work. Any breakdown in communication means that the report has failed. The following functional analysis of the parts of the report is suggested to aid in organizing and presenting the results of scientific and experimental efforts.

- I. Introduction
  - A. Purpose of the investigation (why the work was done)
  - B. How the problem expands/clarifies knowledge in the general field
  - C. Review of related literature
  
- II. Experimental procedure (how the work was done)
  - A. Brief discussion of experimental apparatus involved
  - B. Description of the procedure used in making the pertinent observations and obtaining data
  
- III. Data (what the results were)
  - A. Presentation of specific numerical data in tabulated or graphic form
  - B. Observations made and recorded and all pertinent observations made that bear on the answer to the problem being investigated
  
- IV. Conclusions (final contributions to knowledge)
  - A. General contributions the investigations have made to the answer to the problem
  - B. Further investigation suggested or indicated by the work
  
- V. References –should be the **WORKS CITED ONLY** (the literature Sources that are **ACTUALLY CITED** in the paper)
  - A. Items arranged alphabetically by author’s surname
  - B. References, presented in this order
    1. Author (surname, with initials only)
    2. Date, in parentheses
    3. Title, capitalize first word only
    4. Source: (periodical) (NO ABBREIVATIONS)  
(book) city, state of publication, publisher.

Each item in the Works Cited **MUST ALSO BE CITED WITHIN THE TEXT** of the student paper, using the parenthetical format of the APA Style Manual. Plagiarism is a serious offense, and is **not** limited to direct quotations. Any word, thought, statement, or instruction written by another author and used in the student paper must be appropriately cited in the student paper presented to the Junior Academy.

**Submission of the Report.** Each report must bear an OFFICIAL COVER SHEET, which may be obtained in advance from:

Director of the Tennessee Junior Academy of Science  
Dr. Jack Rhoton  
Box 70301, East Tennessee State University  
Johnson City, TN 37614  
E-mail: [RhotonJ@etsu.edu](mailto:RhotonJ@etsu.edu)

The ORIGINAL COPY of the report should arrive on or before March 1, 2011. The parts of each report should be stapled or clipped, not bound. Heavy covers increase the cost of postage. The student should keep a copy of the report; the original cannot be returned. (We **MUST** have the **ORIGINAL** of all papers –and illustrations- for publication.)

**Selection of the Report.** Each report submitted must be endorsed by a local science or mathematics teacher. The teacher should approve the report as the first member of a selection committee. IT SHOULD BE APPROVED ONLY IF IT IS OF HIGH QUALITY AND REPRESENTS THE STUDENT’S OWN WORK IN RESEARCH AND PREPARATION. The science or math faculty submitting two or more papers in a given category will be asked to serve as judges for those papers and rate them in the order of 1, 2, 3, 4, etc., according to merit before submission to the Tennessee Junior Academy of Science for final judging. The report will then be read by a committee of two or more additional scientists in the field appropriate to the report. Reports will be selected on the basis of research design (30 points), creative ability (20 points), analysis of results (20 points), grammar and spelling (20 points), and general interest (10 points).

**TENNESSEE JUNIOR ACADEMY OF SCIENCE CALENDAR FOR 2010**

March 1	Final Date for Receiving Reports
March 20	Completion of Report Evaluation
March 30	Mailing of Invitations
April 15	Annual Meeting – Nashville (tentative)

**RESEARCH GRANTS FOR SCIENCE PROJECTS  
BY HIGH SCHOOL STUDENTS**

The Tennessee Academy of Science has available a limited number of small research grants (\$100-\$300 per student) to assist high school students involved in developing scientific projects for the TJAS program. These grants are intended to be need-based.

That is, we want to support good proposals from motivated students of adequate ability, where lack of some outside financial support might result in a poor project or possibly no project at all. These grants should not be regarded as competitive merit awards for outstanding proposals or outstanding students, and should not be given to students whose families, or whose project mentors, can readily provide the resources needed. For instance, a project being conducted under the mentorship of a university professor would not, in general, be a good choice for a TAS grant, no matter how able the student and how good the proposed project. It is intended that the TAS research grants program create opportunities for adequately motivated students with access to limited resources to conduct significant, competitive projects. The Tennessee Academy of Science will depend on the sponsoring science or math teachers to provide input into the decision-making process as it concerns the need of applying students and worthiness of their proposed projects.

The application form for the TAS research grant included in these materials may be duplicated as needed. Please note the deadline for receiving grant applications is NOVEMBER 15, 2011. However, the earlier grant applications are received, the sooner grant application funds can be distributed. If you desire further information concerning the TAS research grants program, please write to Dr. Jack Rhoton, Division of Science Education, Box 70301, East Tennessee State University, Johnson City, TN 37614 or E-mail: [RhotonJ@ETSU.edu](mailto:RhotonJ@ETSU.edu).

**TENNESSEE JUNIOR ACADEMY OF SCIENCE**  
**SPRING MEETING - 2011**

The Sixty-Ninth Annual Meeting of the Tennessee  
Junior Academy of Science will be held in  
Nashville, April 15, 2011

All Tennessee high schools are invited to participate in the TJAS program leading up to the spring meeting. The program provides state-wide and national recognition for high school students' investigative or research-type science projects

**TENNESSEE JUNIOR ACADEMY OF SCIENCE REGULATIONS**

The following regulations have been developed to govern the Tennessee Junior Academy meeting by the Standing Committee on Junior Academies of the Academy Conference. Papers must be of a research problem type, with evidence of creative thought. Papers presented should be suitable for publication (typewritten, double-spaced, one side of paper only, name and address on each sheet) and between 1500 and 2000 words in length. Oral presentation will be limited to 10 minutes. Projectors and other audiovisual equipment will be available. Questions on paper presentation will be limited to 3 minutes. All papers should be postmarked **NO LATER THAN MARCH 1, 2011**, and sent to Dr. Jack Rhoton, PO Box 70301, East Tennessee State University, Johnson City, TN 37614. Certificates will be presented to all participants. Sponsoring schools or clubs should have



insurance coverage to protect school participants. The Tennessee Junior Academy of Science can assume no responsibility in this matter.

### **WHAT YOU CAN DO NOW**

If there is no science club at your high school, why not start one? A science club will provide many opportunities to work on problems that will be fun and relaxing. The ready, mutual exchange of ideas can provide a challenging experience in proposing, designing, and completing research into the unknown. Begin now to work on a scientific project to present at the next annual meeting of your local, state, and national Junior Science Clubs. For further information on the Junior Academy program, contact:

Tennessee Junior Academy of Science  
Dr. Jack Rhoton, Director  
PO Box 70301  
East Tennessee State University  
Johnson City, TN 37614  
Phone: 423-439-7589  
E-mail: [RhotonJ@etsu.edu](mailto:RhotonJ@etsu.edu)  
Fax: 423-439-7530

### **PURPOSE OF THE ACADEMIES OF SCIENCE**

The purpose of the various state and municipal Junior Academies is to promote science as a career at the secondary school level. The basic working unit is the science club or area in each school where the extracurricular science projects and activities are supervised by science teachers/sponsors. The American Junior Academy serves a state or city organization much the same as do the professional societies, and it functions in a similar manner; e.g., holding annual meetings for presenting research papers. The parent sponsor of a Junior Academy of Science is the State Academy of Science. The primary activity of the American Junior Academy of Science is the Annual Meeting held with the Annual Meeting of the American Association for the Advancement of Science and the Association of Academies of Science. Top young scientists in each state or city academy are encouraged to present papers and exchange research ideas at the national level. Tours and social hours are also arranged.

## DIRECTORS OF THE TENNESSEE JUNIOR ACADEMY OF SCIENCE

1942-2011

The Tennessee Academy of Science has been the sponsor of the Tennessee Junior Academy of Science since its initial organizational meeting on the Vanderbilt University campus in 1942.

The Directors of the Junior Academy of Science since 1942 are as follows:

- Dr. Frances Bottom – 1942-1955..... George Peabody College  
Nashville
- Dr. Woodrow Wyatt – 1955-1958.....The University of Tennessee  
Knoxville
- Dr. Myron S. McCay – 1958-1963.....The University of Tennessee  
Knoxville
- Dr. Robert Wilson – 1963-1965.....The University of Tennessee  
Chattanooga
- Dr. John H. Bailey – 1965-1976.....East Tennessee State University  
Johnson City
- Dr. William N. Pafford – 1976-1992..... East Tennessee State University  
Johnson City
- Dr. Jack Rhoton – 1992-present.....East Tennessee State University  
Johnson City

**TENNESSEE JUNIOR ACADEMY OF SCIENCE**

Sponsored by the  
**TENNESSEE ACADEMY OF SCIENCE**

**Annual Meeting**

**Belmont University  
Nashville, Tennessee  
April 16, 2010**

*PROGRAM*

9:00 –9:30 a.m.	Registration
9:30 – 9:40 a.m.	Welcome
9:40 –11:30 a.m.	Paper Presentations
11:35 a.m.	Special Presentations
12:00 –1:00 p.m.	Lunch
1:30 –4:00 p.m.	Paper Presentations
4:00 p.m.	Adjournment

**TENNESSEE  
JUNIOR ACADEMY OF SCIENCE**

*Papers to be Presented  
Title of Paper, Student's Name, School, and City*

**THE KINETICS OF ETHANOL PRODUCTION DURING  
FERMENTATION OF A VARIETY OF HIGH-STARCH  
PLANTS**

*Bio-Chemistry*

Ashley Brooke Fuqua  
Greenbrier High School, Greenbrier

**DOPED CADMIUM SULFIDE AS A PHOTOCATALYST FOR  
WATER DECOMPOSITION-A SECOND YEAR STUDY:  
REACTION KINETICS**

*Chemistry*

Gavin Brent Nixon  
Greenbrier High School, Greenbrier

**IDENTIFICATION IN APOLIPOPROTEIN B mRNA- EDITING CATALYTIC POLYPEPTIDE-LIKE 3 F CORE LOCALIZATION SIGNAL** *Immunology*

Hau Phan, Chisu Song, Lorraine Sutton, John P. Donahue, & Richard T. D'Aquila  
Hume-Fogg Academic Magnet School, Nashville

**NEST PREDATION IN THE MURFREESBORO FLAT ROCK CEDAR GLADES, USING HEIGHT AND COLOR VARIABLES** *Biology*

Caitlin Levi & Nealy Pistole  
Siegel High School, Murfreesboro

**ALGAE TOLERANCE IN THE HIWASSEE RIVER** *Environmental Sciences*

Hannah Johnson & Felicity Swafford  
Cleveland High School, Cleveland

**EFFECTS OF PHYSICAL STRESS-INDUCED SLEEP DEPRIVATION ON ENERGY STORES IN** *Biology-Biochemistry*

*Drosophila melanogaster*

Katherine E. Benson  
The University School, Johnson City

**EFFECTS OF AGE ON HABITUATION IN MICE** *Biology*

Thomas Koen  
Pope John Paul II High School, Hendersonville

**EFFECTS OF HABITUATION RATE ON *C. elegans* KNOCKOUTS USING MECHANICAL AND HEAT STIMULI** *Biology*

Kevin Roman & Aziza M. Hart  
School for Science and Math at Vanderbilt, Nashville

**IMPLEMENTATION OF A TRYCYCLIC ANTIDEPRESSANT ON *C. elegans* MUTANTS AND ITS EFFECTS ON LOCOMOTION** *Biology*

Christopher Peek, Alan Herrera, Noel Richards  
School for Science and Math at Vanderbilt, Nashville

**FACTORS THAT INFLUENCE THE DECOMPOSITION PROCESS** *Environmental Sciences*

Jessica Huang  
Clarksville Academy, Clarksville

**THE DISSOLUTION RATE OF NON-NARCOTIC ANALGESICS TO DETERMINE EFFICACY UNDER SIMULATED STOMACH CONDITIONS** *Medicine*

Ashley Elain Corson  
Greenbrier High School, Greenbrier

- HOW THE GROWTH RATES OF *Phaseolus lunatus* AND *Zea mays var ravgosa* ARE AFFECTED BY DIFFERENT INORGANIC AND ORGANIC FERTILIZERS** *Botany*  
Stephanie Surgener & Caroline Elbaum  
Siegel High School, Murfreesboro
- THE EFFECTS OF TEMPERATURE, CONCENTRATION, AND pH ON LACTASE EFFICIENCY** *Chemistry*  
Christine Choo  
Siegel High School, Murfreesboro
- HOW DOES THE ABSENCE OF LIGHT AFFECT THE BIOLOGICAL CLOCKS OF COMMON TENNESSEE CRICKETS?** *Zoology*  
Rezzan Hekmat, Rita E. Pfeiffer, & Anh Pham  
School for Science and Math at Vanderbilt, Nashville
- LINKING FOOD AND MOOD: INSULIN REGULATION OF THE NOREPINEPHRINE TRANSPORTER** *Medicine and Health*  
Vidiya Sathananthan  
Martin Luther King Academic Magnet High School, Nashville
- AQUATIC ECOTOXICOLOGICAL TEST USING PLANARIA** *Chemistry*  
Ryan Kilpatrick & Thomas Griffin  
Pope John Paul II High School, Hendersonville
- FACT RETENTION AS AFFECTED BY VARIED MEDIA** *Behavioral Sciences*  
Elizabeth Coleman  
Northwest High School, Clarksville
- CYRSTALLIZATION WITH VIBRATIONS** *Physics*  
Della Coleman  
Northwest High School, Clarksville
- INHIBITION OF ZEBRAFISH CYCLOOXYGENASE BY NONSELECTIVE AND COX-2 SELECTIVE INHIBITORS** *Molecular Biology*  
Tim Clavin  
Pope John Paul II High School, Hendersonville
- EFFECTS OF SEX AND DOMINANCE ON FREQUENCY OF SENTRY DUTY IN CAPTIVE *Suricata suricatta*** *Zoology*  
Anna C. Brakefield, Logan Shirley, Brittainy Tidwell  
School for Science and Math at Vanderbilt, Nashville

**DETERMINING THE EFFICIENCY OF THE EPA'S  
RAPID BIOASSESSMENT PROTOCOL FOR  
NASHVILLE, TENNESSEE**

*Environmental Sciences*

DeAndre Q. Baynham, Elijah B. Wilson, Eric R. George  
School for Science and Math at Vanderbilt, Nashville

**DETERMINING POTENTIAL INSECT POLLINATORS OF  
*Viola papilionacea* (COMMON VIOLET)**

*Botany*

Hannah R. Asbell, Yi-Ting Huang, & Katherine Roland  
The School for Science and Math at Vanderbilt, Nashville

**RELATIONSHIPS AND EFFECTS OF  
SEDIMENTATION AND CHANNELIZATION OF  
MOUSE CREEK**

*Environmental Sciences*

**(Bradley County, Tennessee)**

Lillie K. Brown  
Cleveland High School, Cleveland

**NURSING NUANCES: JUVENILE TO GERIATRIC  
AND GENDER**

*Behavioral Sciences*

Emily Travis  
Northwest High School, Clarksville

## **Students Who Submitted Papers to the Tennessee Junior Academy of Science**

Abbott, Jamie; Northwest High School, Clarksville  
Asbell, Hannah; School for Science and Math at Vanderbilt, Nashville  
Bailey, Alisha; Northwest High School, Clarksville  
Barry, Susannah; Northwest High School, Clarksville  
Baynham, DeAndre; School for Science and Math at Vanderbilt, Nashville  
Benson, Katherine; University School, Johnson City  
Bentley, Courtney; Northwest High School, Clarksville  
Berget, Nathan; Northwest High School, Clarksville  
Berry, Ga; Northwest High School, Clarksville  
Billings, Tylor; Northwest High School, Clarksville  
Bisgaard, Ethan; Northwest High School, Clarksville  
Bolden, Isaiah; School for Science and Math at Vanderbilt, Nashville  
Brakefield, Anna; School for Science and Math at Vanderbilt, Nashville  
Braley, Eric; Northwest High School, Clarksville  
Britcher, Jennifer; Northwest High School, Clarksville  
Brown, Lillie; Cleveland High School, Cleveland  
Brown, Andre; Northwest High School, Clarksville  
Brown, Ryan; Northwest High School, Clarksville  
Carrington, Brittany; Greenbrier High School, Greenbrier  
Caviness, Lisa; Northwest High School, Clarksville  
Choo, Christine; Siegel High School, Murfreesboro  
Clavin, Tim; Pone John Paul II High School, Hendersonville  
Coleman, Elizabeth; Northwest High School, Clarksville  
Coleman, Della; Northwest High School, Clarksville  
Comeaux, Darryel; Northwest High School, Clarksville  
Corcelle, Ann Marie; Northwest High School, Clarksville  
Corson, Ashley; Greenbrier High School, Greenbrier  
D'Aquila, Richarhd T; Hume-Fogg Academic Magnet High School, Nashville  
Dalton, Amanda; Cleveland High School, Cleveland  
Denley, Emma; Clarksville Academy, Clarksville  
Derickson, Kristina; Northwest High School, Clarksville  
Donahue, John P.; Hume-Fogg Academic Magnet High School, Nashville  
Elbaum, Caroline; Siegel High School, Murfreesboro  
Erickson, Daniel; Northwest High School, Clarksville  
Erwin, Alyssa; Northwest High School, Clarksville  
Forest, Katie; Northwest High School, Clarksville  
Fox, Austin; Greenbrier High School, Greenbrier  
Fuqua, Ashley; Greenbrier High School  
Garner, Newton; Northwest High School, Clarksville  
Gentry, Keith; Northwest High School, Clarksville  
George, Eric; School for Science and Math at Vanderbilt, Nashville  
Greaves, Dontrell; Northwest High School, Clarksville

Griffin, Thomas; Pope John Paul II High School, Hendersonville  
Gross, John; Northwest High School, Clarksville  
Guilliams, Ronja; Northwest High School, Clarksville  
Gutierrez, Alberta; Northwest High School, Clarksville  
Harrigan, Georgia; Northwest High School, Clarksville  
Hart, Aziza; School for Science and Math at Vanderbilt, Nashville  
Hawkins, Sanannah; Siegel High School, Murfreesboro  
Hawkins, Ambriell; Northwest High School, Clarksville  
Hekmat, Rezzan; School for Science and Math at Vanderbilt, Nashville  
Henderson, Richard; Northwest High School, Clarksville  
Herrera, Alan; School for Science and Math at Vanderbilt, Nashville  
Hickman, Tori; Northwest High School, Clarksville  
Horton, Ryan; Northwest High School, Clarksville  
Huang, Yi-Ting; School for Science and Math at Vanderbilt, Nashville  
Huang, Jessica; Clarksville Academy, Clarksville  
Irvin, Lydia; Northwest High School, Clarksville  
Johnson, Hannah; Cleveland High School, Cleveland  
Jonas, Cameo; Cleveland High School; Cleveland  
Jones, Shelby; Northwest High School, Clarksville  
Kadakia, Sonia; Clarksville Academy, Clarksville  
Kelly, Ashleigh; Pope John Paul II High School, Hendersonville  
Kilpatrick, Ryan; Pope John Paul II High School, Hendersonville  
Koen, Thomas; Pope John Paul II High School, Hendersonville  
Lastre, Jonathan; Cleveland High School, Cleveland  
Lee, Justin; Northwest High School, Clarksville  
Leslie, Ashley; Northwest High School, Clarksville  
Levi, Caitlin; Siegel High School, Murfreesboro  
Lindsey, Garrett; Northwest High School, Clarksville  
Ma, Cindy; School for Science and Math at Vanderbilt, Nashville  
Ma, Haiyong; Northwest High School, Clarksville  
Maas, Laura; Pope John Paul II High School, Hendersonville  
Maisha, Musarrat; School for Science and Math at Vanderbilt, Nashville  
Martin, Xerlotta; Northwest High School, Clarksville  
Mayer, Ashley; Northwest High School, Clarksville  
Miraldi, Mark; Northwest High School, Clarksville  
Morrison, Wilfred; Northwest High School, Clarksville  
Nixon, Gavin; Greenbrier High School, Greenbrier  
Orbison, Emily; Siegel High School, Murfreesboro  
Peek, Christopher; School for Science and Math at Vanderbilt, Nashville  
Pfeiffer, Rita; School for Science and Math at Vanderbilt, Nashville  
Pham, Anh; School for Science and Math at Vanderbilt, Nashville  
Phan, Hau; Hume-Fogg Academic Magnet High School, Nashville  
Phillips, Eric; Northwest High School, Clarksville  
Pistole, Nealy; Siegel High School, Murfreesboro  
Plate, Isabella; Cleveland High School, Cleveland  
Porter, Desiree'; Northwest High School, Clarksville



Pribble, Wacey; Northwest High School, Clarksville  
Reyes, Anthony; Northwest High School, Clarksville  
Rhodes, Cynthia; Northwest High School, Clarksville  
Richards, Noel; School for Science and Math at Vanderbilt, Nashville  
Roland, Katherine; School for Science and Math at Vanderbilt, Nashville  
Roman, Kevin; School for Science and Math at Vanderbilt, Nashville  
Sathananthan, Vidiya; Martin Luther King Academic Magnet High School, Nashville  
Schaaf, Zackery; Northwest High School, Clarksville  
Sellick, Megan; Northwest High School, Clarksville  
Shirley, Logan; School for Science and Math at Vanderbilt, Nashville  
Simmons, Amanda; Cleveland High School, Cleveland  
Simon, Chandelor; School for Science and Math at Vanderbilt, Nashville  
Song, Chisu; Hume-Fogg Academic Magnet High School, Nashville  
Surgener, Stephanie; Siegel High School, Murfreesboro  
Sutton, Lorraine; Hume-Fogg Academic Magnet High School, Nashville  
Swafford, Felicity; Cleveland High School, Cleveland  
Thorpe, Brittni; Northwest High School, Clarksville  
Tidwell, Brittainy; School for Science and Math at Vanderbilt, Nashville  
Travis, Emily; Northwest High School, Clarksville  
Vielma, Samantha; Northwest High School, Clarksville  
Wallace, Kanika; Northwest High School, Clarksville  
Welborn, Lauren; Northwest High School, Clarksville  
White, Jessica; Clarksville Academy, Clarksville  
Wilson, Elijah; School for Science and Math at Vanderbilt, Nashville

## PAPERS OF EXCELLENCE

### **Identification in Apolipoprotein B mRNA-editing Catalytic Polypeptide-like 3 F Core Localization Signal**

Hau Phan, Chisu Song, Lorraine Sutton, John P. Donahue, & Richard T. D'Aquila  
Hume-Fogg Academic Magnet High School, Nashville

#### **Abstract**

The human apolipoprotein B mRNA-editing catalytic polypeptide-like 3 (APOBEC3) proteins belong to a family of cellular cytidine deaminases. Among this family, APOBEC3G (A3G) and APOBEC3F (A3F) can restrict human immunodeficiency virus type-1 (HIV-1) replication. Both proteins consist of two cytidine deaminase domains- one in the N-terminus and the other in C-terminus. Previous studies demonstrated that A3F and A3G are incorporated into budding virions in the absence of viral infectivity factor of HIV-1. Further analysis demonstrates that while the A3G is distributed both inside and outside the mature-core, A3F is only localized into mature-core of HIV-1. Moreover the core localization signal(s) of A3F is demonstrated to be at the C-terminal domain of A3F. In this study we tried to characterize the core localization signal through site-directed mutagenesis. By taking advantage of the facts that viral incorporation patterns of another A3 proteins, A3C, is like that of the A3G while the C-terminal domain of A3C is almost identical with C-terminal domain of A3F we identify two potential signal motifs and introduce substitution as well as deletion mutations into the potential motifs respectively. We hypothesize one of these sites might play a role as the signaling sequence motif for core localization of A3F.

## Introduction

Human genome encodes a group of cellular cytidine deaminases called *apolipoprotein B* mRNA-editing catalytic polypeptide-like (APOBEC). Two of the seven members of APOBEC3 proteins have proven to be powerful inhibitors of the Human Immunodeficiency Virus type 1 (HIV-1) replication *in vivo* - APOBEC3F (A3F) and APOBEC3G (A3G) (1,2). However, wild-type HIV-1 virus encodes virion infectivity factor (Vif) that counteract the antiretroviral effect of the A3F and A3G through degradation of the proteins (6).

In Vif-deficient viruses, A3G and A3F are incorporated into budding viral particles and inhibit its replication in the next target cell (4, 5). Through the cytidine deaminase activity of the proteins, A3F and A3G deaminate the cytidines into uridines in the minus-strand cDNA during reverse transcription process, thus mutating the complementary strand from G-to-A of HIV-1 cDNA. These mutations either introduce fatal mutations or incorrect stop codons, rendering the viral DNA useless, and therefore, inhibiting replication.

Though A3F and A3G display well conserved amino acid sequences in their N-terminal domain, the two are very different when it comes to encapsidation into budding virion (unpublished data). A3F is incorporated into the mature viral core while A3G is incorporated both inside and outside the mature viral core of HIV-1 (7). Furthermore, it has been demonstrated that C-terminal domain of A3F protein plays a critical role for its encapsidation into mature viral core (unpublished data). Single domain A3C, another member of the APOBEC family, displays highly conserved amino acid sequences with the C-terminal A3F while the distribution patterns of the A3C in the mature virions is like that

of the A3G (3). Because it behaves like A3G in the mature virions but contains almost identical amino acid sequences with the C-terminal domain of A3F, A3C is a perfect working model to investigate core localization signal(s). Amino acid sequence alignment between A3C and the C-terminal domain of A3F revealed two areas of dramatic amino acid sequences disparity, making these two sequence motifs good potential signal motifs to analyze core localization of A3F (3).

Mutations were introduced into these two potential sites-one at 310 where DTD of A3F were substituted into YPC of A3C and another at 359 where a stop-codon was introduced.

### **Methods and Procedures**

**Site-directed mutagenesis and Plasmids.** pcDNA3.1(-) A3F, the A3F expression plasmid, was used as a template to introduce mutations into the potential signal motif sites. To introduce mutations into wild-type A3F coding sequences, the following primers were used:

For DTD311 - 313 YPC amino acid substitutions;

Forward primer 5' – CTACTACTTCTGGTATCCATGTTACCAGGAGGGGCT – 3'

Reverse primer 5' – GAGCCCTCTCTGGTAACATGGATAACCAGAAGTAGTA – 3'

To introduce the stop-codon into C-terminal amino acids at the position of 359 of A3F, the following primers were used:

Forward primer 5' – GGACTAAAATACAACACTGACTATTCCTGGACAGC – 3'

Reverse primer 5' – GCTGTCCAGGAATAGTCAGTTGTATTTTAGTCC – 3'

Using the primers, we performed a Polymerase Chain Reaction (PCR) to generate full length pcDNA3.1(-) A3F with the mutations. After the PCR, the resulting DNAs were digested with DpnI restriction enzyme which can only digest the parental DNA, leaving the DNA with mutations intact.

After transforming the mutated DNAs into competent *E. coli* bacteria cells, HB101, the bacteria was plated in 3 ml bacterial culture media. The culture plasmid DNAs were then purified using Ququick miniprep kit. The mutation into the A3F plasmid DNAs were confirmed using DNA sequencing analysis.

### Data and Results

```

                190         200         210         220         230
A3F-C          LKEILRNPM EAMYPHI FYFHFKNLRKAYGRNESWLCFTMEVVKHHSPISW
A3C            MNPQIRNPMKAMYPGTFYFQFKNLWEANDRNETWLCFTVEGIKRRSVVSW
                :****  ****   ***  ****   *   ***:***** *  :*: : *  :**

                240         250         260         270         280
A3F-C          KRGVFRNQVDPETHCHAERCFLSWFCDDILSPNTNYEVTWYTSWSPCEC
A3C            KTG VFRNQVDSETHCHAERCFLSWFCDDILSPNTKYQVTWYTSWSPCPDC
                *  *****  *****  *****  *****  *  *****  *****: *

                290         300         310         320         330
A3F-C          AGEVAEFLARHSNVNLTIFTARLYYFWDTDYQEGLRSLSQEGASVEIMGY
A3C            AGEVAEFLARHSNVNLTIFTARLYYFQYPCYQEGLRSLSQEGVAVEIMDY
                *****  *****  *****  *****  *****: ***** *

                340         350         360         370
A3F-C          KDFKYCWENFVYNDDEPFKPKWGLKYNFLFI DSKLQEILE
A3C            EDFKYCWENFVYNDNEPFKPKWGLKTNFRLI KRRLRESLQ
                *****  *****  **   *   : * * *

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The amino acid sequence alignment of the C-terminal of A3F and A3C shows two areas of dramatic difference-at site 310-312 and at 360-the end (boxed areas)(3). The A3C is incorporated into both inside and outside of mature virus while A3F is enriched into the mature viral core only. Furthermore, previous study demonstrated the core

localization signal of A3F is located in the C-terminal domain. Therefore, it is hypothesized that one of these two amino acid motifs might play the role as the signal motif for the core localization of A3F during viral incorporation of HIV-1.

When the mutant of A3F with the DTD to YPC substitutions at the position of 310 was analyzed by lab members, the mutation had no effect on the distribution of the mutant A3F proteins in the mature virions indicating that the mutant A3F was still incorporated into the virion core and restrict HIV-1 like that of the wild-type A3F (data not shown).

Next the C-terminal stop-codon mutant at the position of 359 of A3F was analyzed. Preliminary data indicated that C-terminal stop-codon mutant of A3F display quite distinctive distribution of the protein mainly found outside the mature viral core while wild-type A3F is found only in mature viral core.

### **Discussion and Conclusions**

A3F and A3G proteins play a critical role in inhibiting HIV-1 replication. However the underlining biological mechanism for their effects on HIV-1 restriction is not well understood. Previous studies demonstrate that A3F and A3G are incorporated into budding HIV-1 virion equally well. However further analysis indicate that the modes of incorporation into budding virion between A3F and A3G are quite distinctive, A3F being highly enriched into the mature viral core while A3G being distributed equally both inside and outside the core. In this study we tried to identify the core localization signal for A3F. To identify core localization signal motif(s) in the A3F, two potential amino acid sequences were identified through sequence alignment between A3C and C-terminal A3F (3).

After identifying the two potential signaling motifs, mutations were introduced at site 310- from DTD to YPC of A3F by employing site-directed mutagenesis using PCR. The resulting mutant A3F was tested for its effect on the core localization of the proteins in the HIV-1 particles. The data indicated that the DTD to YPC mutation has no effect on the core localization and antiretroviral effect of the protein against HIV-1, indicating that the DTD sequences are not the core localization signal motif.

Preliminary data by other lab members using the second mutant into the C-terminal end stop-codon indicate that the majority of the mutant A3F is localized outside the core unlike that of the wild-type A3F and the antiretroviral activity of the mutant A3F is compromised. These data strongly indicated that the second motif might be responsible for core localization of A3F. Further experiments are underway to corroborate the preliminary data

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# **The Kinetics of Ethanol Production During Fermentation of a Variety of High-Starch Plants**

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## **Abstract**

Because of the concerns about the increase of fuel prices and atmospheric warming, ethanol, an alternative energy source, is being extensively studied due to its renewability and its cost effective nature. Ethanol is currently used as a gasoline additive and/or extender. To determine the best choice of yeast to use for this study, seven subspecies of yeast (*S. cerevisiae*) were used to ferment pure dextrose solutions. Fast-rise yeast typically used for baking was the most effective of those tested. The fast-rise yeast was then used with different concentrations of dextrose solutions (0.005 M, 0.010 M, and 0.025 M) to determine the rate law (rate constant,  $k = 2 \times 10^{-5}$ , order = 1).

Irish potatoes (*S. tuberosum*), sweet potatoes (*I. batatas*), turnips (*B. rapa rapa*), beets (*B. vulgaris vulgaris*) and radishes (*R. sativus*) were used with the fast-rise yeast to determine the plant species that would have the highest ethanol yield (1). Sweet potatoes produced the highest concentration of ethanol in the shortest time period ( $1.03 \pm 0.08 \times 10^{-6}$  moles $\cdot$ s $^{-1}$ ).

## **Introduction**

Due to the concerns about the increase of fuel prices and atmospheric warming, alternative sources of fuel are being extensively studied. Because it is one of the most cost effective alternatives, ethanol from renewable sources is one that is being seriously considered. Ethanol is a high octane fuel that is currently used as a gasoline additive

and/or extender (8). Initially, Henry Ford designed the Model T to run on alcohol as he believed it to be the fuel of the future (9).

Ethanol has been produced from sources such as corn, sugarcane (*saccharum officinarum*), and switchgrass (2). However, certain other high-starch plants are being investigated for the amount of ethanol they yield. Sweet potatoes (*ipomoea batatas*) have recently been investigated as material to use based on certain USDA studies that have concluded that sweet potatoes did as well if not better than corn in the amounts of ethanol produced (3). Other materials investigated were irish potatoes (*solanum tuberosum*), sugar beets (*beta vulgaris vulgaris*), turnips (*brassica rapa rapa*), and radishes (*raphanus sativus*) (10). Also, citrus peels have been found to quickly convert to alcohol. Due to the abundance of dried citrus peels from the existing citrus industry, this would be an ideal opportunity due to the abundance of this waste (~500 tons of waste per day) (6). The possibility of sugarcane being used for ethanol production in the United States is incredibly unlikely due to the high costs and the climate requirements. However, it has enjoyed a huge success in Brazil (7).

Sweet potatoes might be more difficult to commercialize due to high labor and costs involved in cultivation. But an advantage for sweet potatoes is that they require much less fertilizer and pesticide than other plant materials (3). It has been found that five tons of sweet potatoes could be produced per acre in Maine, compared to a mere 2.5 tons of corn (another alternative) per acre produced in Maine (3).

Enzymes enable simple sugars to be available to the fermentation yeast. Therefore, they expedite the fermentation process, and they also make it easier to separate liquid from solids after the ethanol has been produced. Certainly, the more efficiently one separates

the free liquid from the solids, the more energy efficient the process can be (4). The major obstacle to ethanol production is in finding new enzymes that can break down tough cellulose (5). Research has found that adding certain enzymes during fermentation speed up the break-down process and increase ethanol yields (4).

This research project was done in order to determine the yeast and plant varieties that produce the highest ethanol yields. Since it is simpler to measure carbon dioxide pressure than ethanol during fermentation, this method was used to calculate the reaction kinetics.



The pressure, volume, and temperature of the  $\text{CO}_2$  were monitored, and the ideal gas law was used to calculate the number of moles of  $\text{CO}_2$ . The  $\text{CO}_2$  and ethanol were produced in a one-to-one ratio to allow the calculation of ethanol production rate from the  $\text{CO}_2$  rate.

### **Methods and Procedures**

*Determining the most effective yeast (*S. cerevisiae*) variety:*

10 g of glucose was dissolved in 100 mL of deionized water prepared with a standard Barnstead Deionization System. 1.0 g separately of each yeast variety was then mixed with this solution. The mixture was placed into an apparatus (Figure 1) with a total volume of 440 mL equipped with a Vernier Gas Pressure sensor connected to the appropriate Vernier software. The device was sealed with Teflon tape to ensure an airtight seal, and pressure data were collected at a rate of ten samples per hour for fifteen hours. The temperature remained constant at 22<sup>0</sup>C for the duration of the data collection.

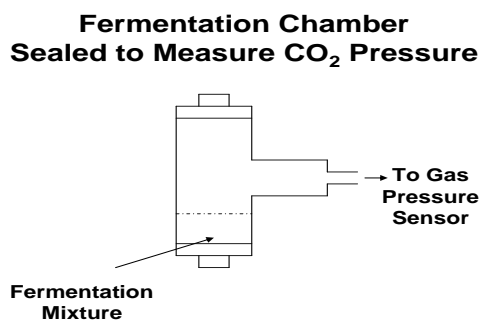
*Method of initial rates:*

100 mL of glucose at concentrations of 0.005 M, 0.010 M, and 0.025 M was reacted with 1.0 g of fast-rise yeast (selected from the seven types tested). Data were collected at the same rate as above, ten samples per hour for fifteen hours. These data were then used to determine the rate law for the reaction:  $\text{Rate} = k[\text{CH}_3\text{CH}_2\text{OH}]^x$ .

*Rates for the high-starch plants:*

100 grams of each sample of plant material was cubed, added to 200 mL of DI water, and pureed with a commercial blender on high. 100 g of this slurry was placed into the apparatus with 1.0 g of fast-rise yeast. The apparatus was sealed and data were collected at a rate of ten data points per hour for fifteen hours as described above. For those samples that reached a maximum measurable pressure before complete conversion to ethanol (sweet potatoes and beets), a second run was conducted using 50 g of sample

**Figure 1**



## Data and Results

Figure 2 shows the rate of reaction for a variety of *s. cerevisiae* subspecies. These varieties have typically been used in wine production and baking processes. Of those tested the fast-rise yeast used in baking proved to be best at an ethanol production rate of  $0.0500 \text{ moles}\cdot\text{s}^{-1}$ . The other subspecies tested were GRE, BA11, BM45, 71B, DV10, and baker's yeast. All remaining experiments were performed using the fast-rise variety to maximize ethanol production rate. Rate calculations were done using the change in pressure of the  $\text{CO}_2$  ( $\Delta P_{\text{CO}_2}$ ) within the reaction vessel for a 2-4 hour period after the curve became linear. This result along with the head volume (V) in the apparatus and the temperature (T) were used to calculate the number of moles of  $\text{CO}_2$  which translated to moles of ethanol.

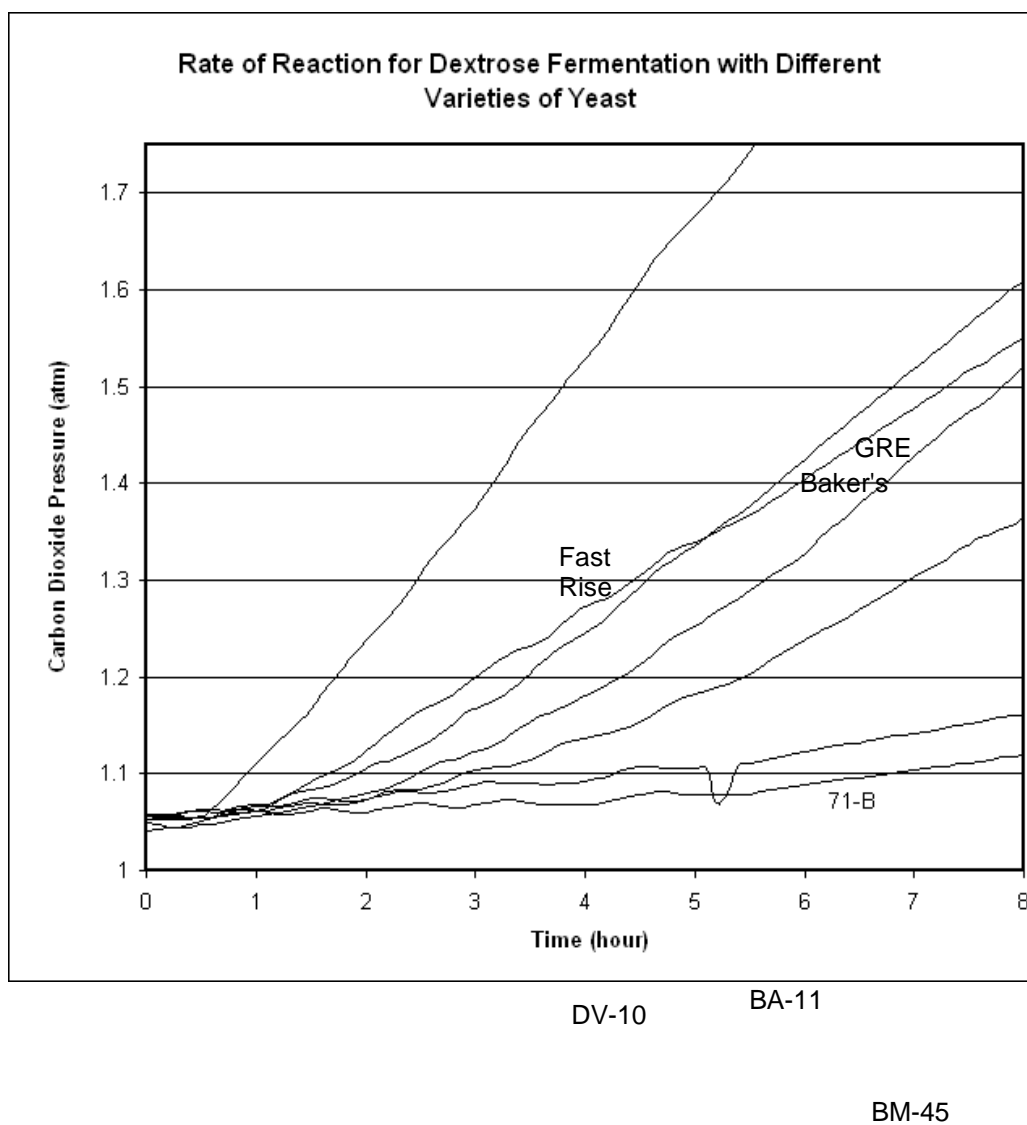
$$\text{Since: } (\Delta P_{\text{CO}_2}) V = (n_{\text{CO}_2}) RT$$

$$\text{Then: } n_{\text{CO}_2} = n_{\text{Ethanol}}$$

$$\text{Rate} = \frac{n}{t}$$

Where n = number of moles of ethanol produced over the selected time span. The lowest fermentation rate came from the yeast type 71B at a rate of  $0.0014 \text{ moles}\cdot\text{s}^{-1}$ . The others ranged from  $0.0109$  to  $0.0257 \text{ moles}\cdot\text{s}^{-1}$  as given in Table 1.

Figure 2

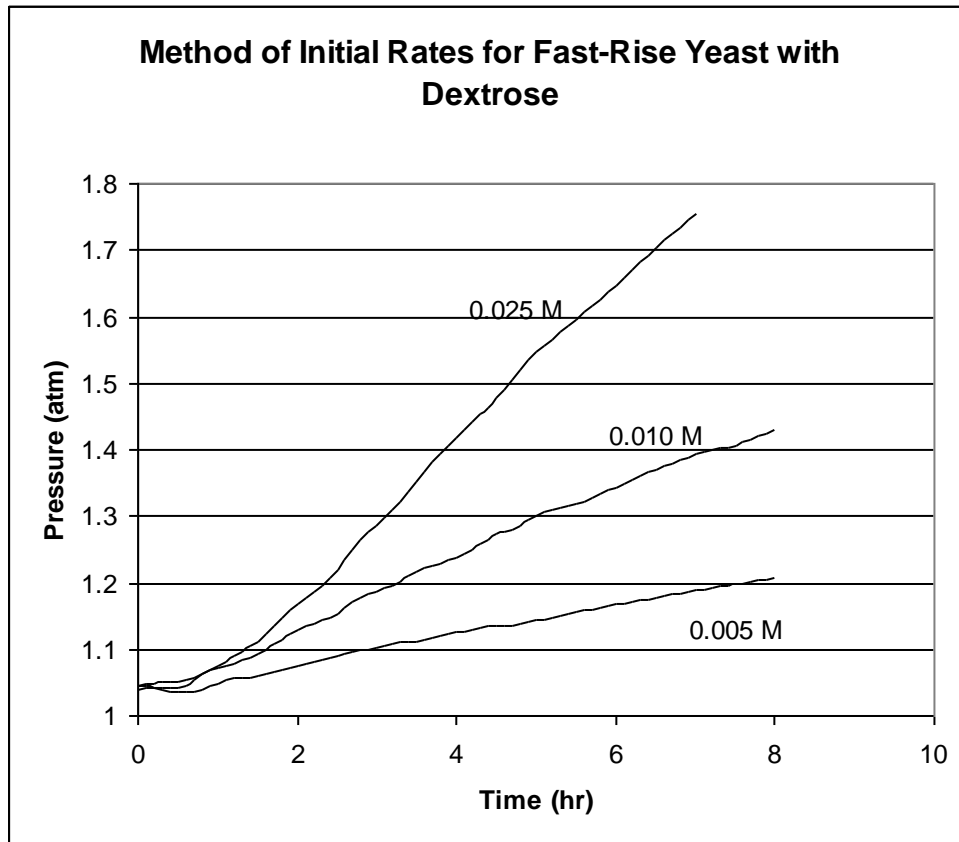


**Table 1**

Yeast Type	Ethanol Production Rate (mol·s <sup>-1</sup> )
GRE	0.0242
BA11	0.0109
BM45	0.0033
71B	0.0014
DV10	0.0257
Bakers	0.0173
Fast Rise	0.0500

As shown in Figure 3 fast-rise yeast was used with different concentrations of dextrose solutions (0.005 M, 0.010 M, and 0.025 M) to determine the rate law. The rates increased from a 0.005 M solution with a rate of  $0.939 \times 10^{-7} \text{ mol} \cdot \text{s}^{-1}$  up to the 0.025 M solution with a rate of  $4.75 \times 10^{-7} \text{ mol} \cdot \text{s}^{-1}$ , increasing by five times. The data from Table 2 were then used by the method of initial rates to calculate the rate constant to be  $2 \times 10^{-5}$  and the reaction order to be 1.

**Figure 3**



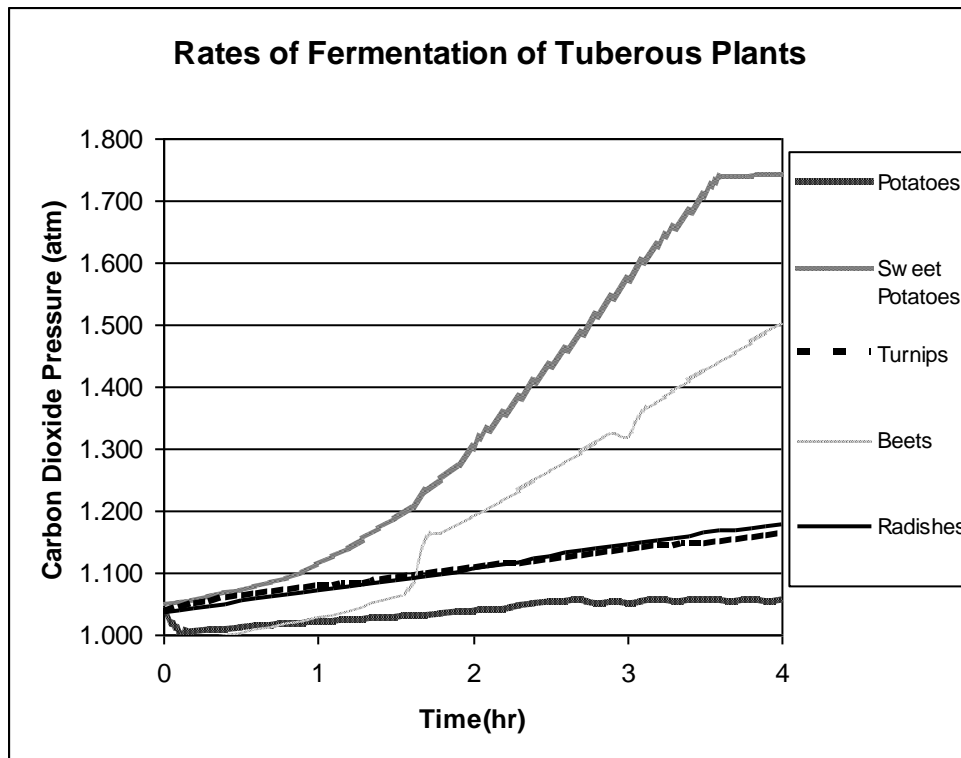
**Table 2**

Method of Initial Rates-Ethanol Production by Fermentation of Dextrose by Fast Rise Yeast	
C (Dextrose)(M)	Rate (mol/s)
0.005	$0.939 \times 10^{-7}$
0.010	$2.07 \times 10^{-7}$
0.025	$4.75 \times 10^{-7}$



Sweet potatoes (*I. batatas*) produced the greatest amount of ethanol in the shortest amount of time. Therefore, sweet potatoes would be the most efficient commercial crop for ethanol production. Irish potatoes would be the worst choice because during the experiments performed in the course of this research the carbon dioxide pressure never exceeded 1.1 atmospheres during the time window of reaction. The second highest producer of ethanol was beets (*B. vulgaris vulgaris*), which fluctuated during the course of the experiment. However, the carbon dioxide pressure exceeded 1.5 atmospheres, 0.3 atmospheres less than the sweet potatoes. The intermediate results were for radishes and turnips, which paralleled each other during the runs (Figure 4).

**Figure 4**



**Table 3**

Ethanol Production by Fermentation Rates for High Starch Plants	
Type	Rate
solanum tuberosum	$4.79 \times 10^{-8}$
ipomoea batatas	$1.03 \times 10^{-6}$
brassica rapa var. Rapa	$1.12 \times 10^{-7}$
Beta vulgaris vulgaris	$4.90 \times 10^{-7}$
raphanus sativus	$1.54 \times 10^{-7}$

**Conclusions**

Fast rise yeast was the overall best choice for ethanol production due to its quick action. Therefore, this variety of yeast was used for the remaining experiments.

According to a recent study by the United States Department of Agriculture, 5.7 tons of sweet potatoes could be produced per acre of land in Maryland (3). In order for this source to replace a significant portion of gasoline to aid the economy as a primary fuel, it needs to provide twenty-five or more percent of required fuel volume. Using the 5.7 tons of sweet potatoes per acre as the standard this research shows that one acre of land would be able to produce 14.2 gallons of pure ethanol per growing season. So, for one car with a tank size of 16 gallons, 1.2 acres of sweet potatoes would be needed per fill up.

### **Future Direction**

Research could be done on ethanol production with a wider range of plant life than the narrowed scope of high-starch tuberous plants. For example, the main focus in the media for the past several years for ethanol production is corn and sugarcane. Another area of research could be to grow the plants under controlled conditions and to eliminate the possibilities of other variables affecting the growth, for example pesticides or other chemicals that might alter results. Besides ethanol, one could extend the research into biodiesel and compare the pros and cons for both against one another.

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# **Effects of Sex and Dominance on Frequency of Sentry Duty in Captive *Suricata suricatta***

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## **Abstract**

*Suricata suricatta*, more commonly known as the meerkat, is a highly social mammal that spends the majority of its time foraging. While the rest of the colony is foraging, sentry meerkats watch for predators, both in the wild and in captivity. We observed the meerkats at the Nashville Zoo at Grassmere once a week for three weeks and investigated different factors we believed could influence sentry duty. We hypothesized that the male and dominant meerkats of a captive population would perform sentry duty longer and more frequently than females and subordinates. Our data showed that males performed almost twice as many sentry shifts as females, and their shifts were three times longer. The dominant meerkats performed sentry duty more often than the subordinates, but their shifts were shorter. In conclusion, captive male meerkats performed significantly more sentry shifts and spent more time on sentry duty than females. There was no significant correlation between amount of time spent on sentry duty and dominance. By comparing this data to that of wild meerkats, we can determine how captivity affects the meerkats' natural behavior.

## **Introduction**

*Suricata suricatta*, commonly known as the meerkat, is in the mongoose family and is native to southern Africa and the Kalahari Desert (Macdonald and Hoffmann, 2008). In the wild, these small, yellow-brown mammals forage constantly for

scorpions and other desert insects (Fuehrer, 2003). They live in colonies with a dominant pair, which is the only breeding pair (Fuehrer, 2003). They create intricate burrow systems to avoid their natural predators, which include hawks and snakes (Anonymous, 2009). Meerkats also perform sentry duty while others are foraging, and they emit specific alarm calls to alert the others of danger (Fuehrer, 2003). This instinctual action occurs both in nature and in captivity, where there are generally no natural predators present. Because a captive population is easier to observe than a wild population, we chose to study a zoo's meerkat colony to determine which factors influence sentry duty. By looking at the length and frequency of each individual meerkat's time on sentry duty, we were able to analyze whether gender or dominance within the colony impacted a meerkat's sentry duty contribution. We hypothesized that the male and dominant meerkats of a captive population would perform sentry duty longer and more frequently than females and subordinates.

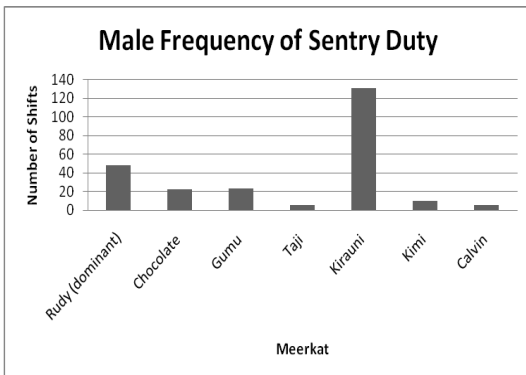
### **Methods**

We visited the Nashville Zoo at Grassmere for three to six hours every Wednesday for three weeks in April and May. The zoo maintains a colony of twelve meerkats in an enclosure which was roughly 50 by 25 feet. The enclosure was filled with soil to allow the meerkats to burrow, and had very little canopy cover, with only three small trees with few leaves. Rocks and logs in the enclosure provided the meerkats with effective vantage points for sentry duty, and for recording purposes we classified these structures as "high ground". Before beginning formal observations, we identified unique characteristics of each meerkat which allowed us to recognize individuals. This was the first time anyone, including the keepers, was able to consistently identify all

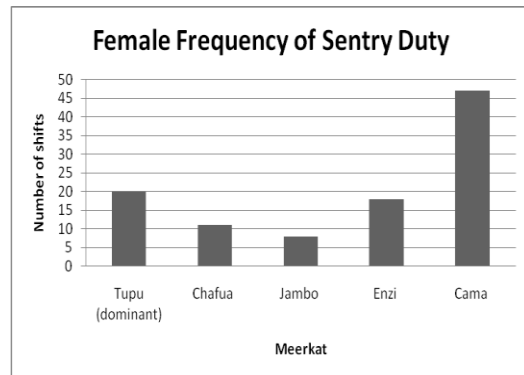
twelve meerkats. We then monitored them and recorded the identity of the meerkat, the length of sentry duty, and the type of sentry duty. After a short observation period, we determined that the meerkat's sentry duty behavior could be classified into three groups; Type 1, standing on high ground, Type 2, sitting on high ground, and Type 3, standing on low ground.

### Results

We then analyzed the data and compared results from males and females as well as dominant and subordinate meerkats. We found that, on average, males performed almost double the number of sentry shifts compared with females ( $p=0.0085$ ) (Figures 1 and 2). The males, both dominant and subordinate, performed a total of 244 sentry duty shifts. The females performed a total of 104 shifts. It should be noted that these numbers are taken from 7 males and only 5 females, although the t-test corrected for this discrepancy.



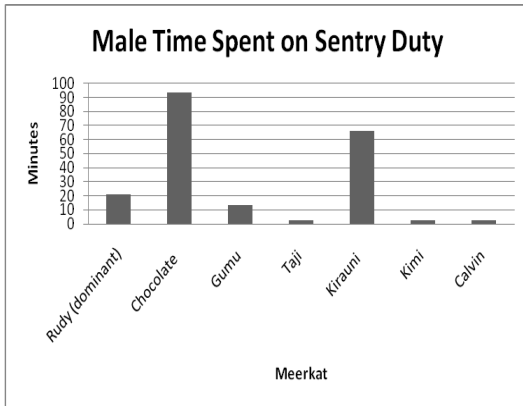
**Figure 1:** The total number of shifts performed by each of the male meerkats during the three week observation period. The dominant male has been noted for comparison.



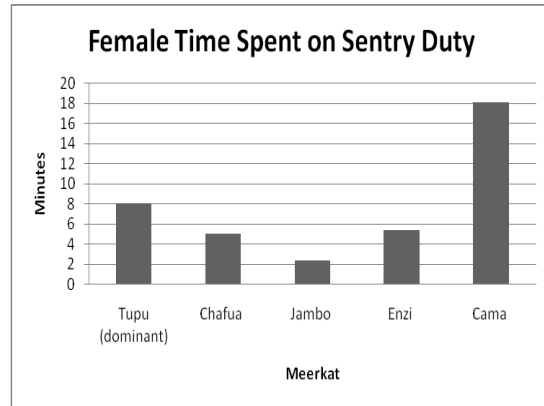
**Figure 2:** The total number of shifts performed by each of the female meerkats during the three week observation period. The dominant female has been noted for comparison.

As well as performing more shifts, males also spent much more time on sentry duty than the females ( $p=0.1807$ ). The males spent a total of 202 minutes on sentry

duty over the three weeks, while the females only spent 40 minutes total on sentry duty. When corrected for the differences in the number of males and females, males performed an average of 29 minutes each and females performed an average of merely 8 minutes each (Figures 3 and 4).



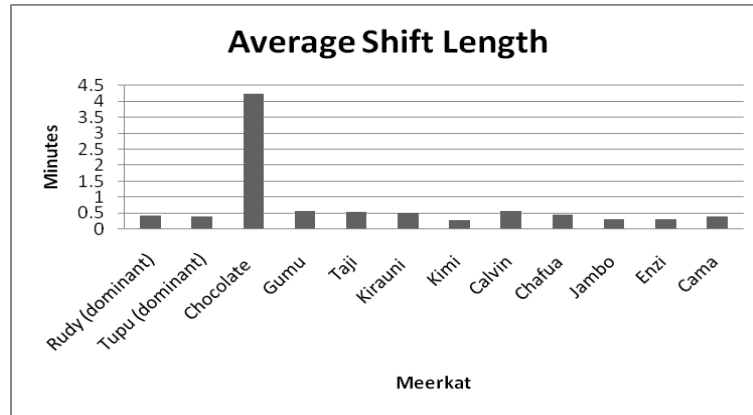
**Figure 3:** The total amount of time on sentry duty by each of the male meerkats during the three week observation period. Notice the outliers Chocolate and Kirauni.



**Figure 4:** The total amount of time on sentry duty by each of the female meerkats during the three week observation period.

The dominant pair performed sentry duty more frequently than the subordinates with the dominant pair performing an average of 34 shifts each and the subordinates performing an average of 28 shifts each. However, the subordinates performed longer shifts than the dominants, although the difference was not significant ( $p=0.692$ ). The subordinates' average total time on sentry duty was 21 minutes over the three weeks, and the dominants' average total time was 14 minutes. This means that the average shift length for subordinates was 0.758 minutes and the average shift length for dominants was 0.425 minutes (Figure 5).





**Figure 5:** The average shift length for each of the meerkats during the three weeks. Notice the outlier Chocolate. The dominants have been noted in parentheses.

The two outliers of the group were Kirauni and Chocolate. Kirauni performed more sentry shifts than the other meerkats, and Chocolate performed much longer shifts than the others. At the end of the three week observation period, Kirauni had performed sentry duty 131 times. The next highest frequency was Rudy, the dominant male, with 48 shifts. Chocolate had the longest amount of time on sentry duty, spending a total of 93 minutes on sentry duty in the observation period. Kirauni followed Chocolate with a total of 66 minutes, and the next highest was Rudy with 21 minutes.

On several occasions, the meerkats’ normal behavior was altered by outside occurrences. The most common of these occurrences was planes flying overhead, which caused the entire colony of meerkats to stop what they were doing and watch the plane until it was gone. At one point during our observations, a seeing-eye dog came to the enclosure. The meerkats lifted their tails to appear larger and joined together to mob the dog. Once the dog had left and the apparent threat was gone, they resumed normal behavior.

## **Discussion and Conclusions**

There was a significant difference in total time spent on sentry duty between male and female meerkats. On average, males had longer sentry shifts than females and performed sentry duty more frequently than females. The results for frequency of sentry duty were statistically significant, but the results for sentry shift length, while not statistically significant, showed a trend toward males performing longer sentry shifts. The dominant pair of meerkats performed more sentry shifts and spent more time on sentry duty than the subordinate meerkats, but their shifts were, on average, shorter. The dominant male meerkat (Rudy) had the second highest frequency of sentry duty and third most time spent on sentry duty among males. The dominant female (Tupu) had the second greatest frequency and time spent on sentry duty. There was no significant correlation between dominance and sentry duty.

Subordinate males were the group that spent the most amount of time on sentry duty and had some of the highest frequencies of sentry duty. Since meerkats perform sentry duty only once they have eaten their fill during foraging, this result suggests subordinate male meerkats may be those most apt at finding food or those most aggressive at foraging. According to our research, captive male meerkats spend significantly more time on sentry duty than females, but there is no significant correlation between amount of time spent on sentry duty and dominance.

Our data were only collected during Wednesday midmornings in the spring, which may have affected the results. Although we spent the week before our research began studying the meerkats in order to properly identify them, misidentification is a possibility. One of the rocks was also a heating rock, which may have led some of the meerkats to perform sentry duty when they otherwise would not have. This situation

was especially possible during the one week in which it was particularly cold. There were also a few times when a Seeing Eye dog or loud vehicle would startle the meerkats and disrupt their behavior. Two particular meerkats, Kirauni and Chocolate, were outliers in their respective fields; Kirauni performed sentry duty almost three times as much as the next leading meerkat, and Chocolate spent more time on sentry duty than almost all other meerkats combined.

Meerkats in the wild usually perform sentry duty only once they have eaten their fill (Clutton-Brock, et al. 1999); if this is also true for captive meerkats, this result suggests subordinate male meerkats may be those most apt at finding food or those most aggressive at foraging. A study of the amount of food consumed by each demographic of captive and wild meerkats would help develop this hypothesis.

### **Acknowledgements**

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# **Linking Food and Mood: Insulin Regulation of the Norepinephrine Transporter**

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## **Abstract**

Norepinephrine (NE) is a neurotransmitter whose signaling is vital to the function of the brain and the body as a whole. Norepinephrine signaling in the nervous system regulates sleep, mood, depression, learning, memory, appetite, attention, and the fight or flight response. Norepinephrine signaling is modulated by the norepinephrine transporter (NET), which is the primary mechanism for the removal of NE from the synapse. Thus NET is critical for regulating both the duration and intensity of NE signaling in the brain, and any disruption of NET function possesses a threat to both autonomic function and mental health. Interestingly, early studies indicate that NET function and activity may be regulated by insulin, a hormone critical for the regulation of metabolism. Thus insulin regulation of NET provides a plausible link between metabolism and mood. In fact the presence of diabetes doubles the risk of comorbid depression, and one quarter of the diabetic population is affected by depression (Lustman and Clouse, 2005). Furthermore, functional deficiencies in the activity of central neurotransmitter, such as norepinephrine and serotonin, have been shown to be evident in animal models of diabetes, and may predispose patients to the development of depression. In these experiments, we seek to determine the role of insulin signaling in modulating NET function. In particular we are interested in whether insulin is capable of regulating NET surface expression and in

determining specific components of the insulin signaling pathway that are involved in this regulation.

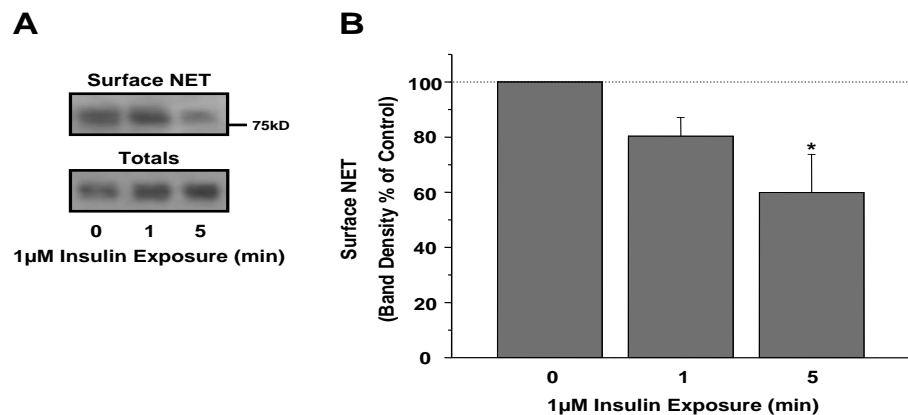
## **METHODS AND MATERIALS**

**Cell culture and transfection:** CHO cells stably transfected with HA tagged human NET (hNET cells) were maintained in Ham's F12 Media/10% FBS/L-Glu/pen/strep. Cells were plated on poly-D-lysine (Chemicon/Millipore, Billerica, MA)-coated plates for each experiment and incubated for 24 to 48 h before each experiment. Transient transfections with Akt-KD, a kinase dead (catalytically inactive) construct of Akt with the lysine at residue 179 replaced with an arginine (AKT-K179R) (Garcia et al., 2005), was graciously provided by Dr. R. Roth Stanford University (Stanford, CA). Transfections were performed with Fugene6 according to manufacturer's instructions (Roche), and experiments on transfected cultures were performed 48 hours following transfection.

**Cell-Surface Biotinylation:** Cell-surface biotinylation was used in order to determine the amount of NET on the surface of the cells after being incubated with different drug treatments. Chinese Hamster Ovary cells (CHO cells), stably expressing human NET (hNET), were plated into six-well plates approximately 48 hours before the experiment. After 1 hour starvation in KRH/glucose solution, the cells were incubated with different concentrations of insulin in KRH/glucose solution at 37° C for varying amounts of time. The cells were washed twice with ice-cold PBS-Ca-Mg and then treated with Sulfo-NHS-SS biotin on ice for 30 minutes. The reaction was quenched by washing twice with PBS-Ca-Mg-glycine solution followed by incubation with PBS-Ca-Mg-glycine for 15 minutes on ice. Cells were then washed twice with PBS-Ca-Mg before lysis with

400 $\mu$ L lysis buffer (200  $\mu$ L 100 mM PMSF, 400  $\mu$ L Triton, 20 mL PBS w/ Ca<sup>2+</sup> and Mg<sup>2+</sup>) for 1 hour on ice with vigorous shaking. The supernatants were isolated (for total protein), and the biotinylated proteins were separated by incubation with streptavidin beads overnight at 4°C with constant mixing. The protein-bead complex was washed three times with lysis buffer. The complex was then eluted with sample buffer (950  $\mu$ L Laemmli sample buffer, 50  $\mu$ L  $\beta$ ME (beta-mercapto-ethanol)) for 5 minutes at room temperature and then 5 minutes at 95°C. Total cell lysates and biotinylated proteins were separated by SDS-polyacrylamide gel electrophoresis (10%) and transferred to PVDF membranes. PVDF membranes were incubated with blocking buffer (TBST+5% milk) for 1 hour at room temperature and then immunoblotted with primary anti-NET antibody and then secondary anti-NET antibody. Immunoreactive bands were visualized using ECL Plus detection system on chemiluminescence film. Band densities were calculated using Scion Image software and normalized to the appropriate total extract to control for protein loading.

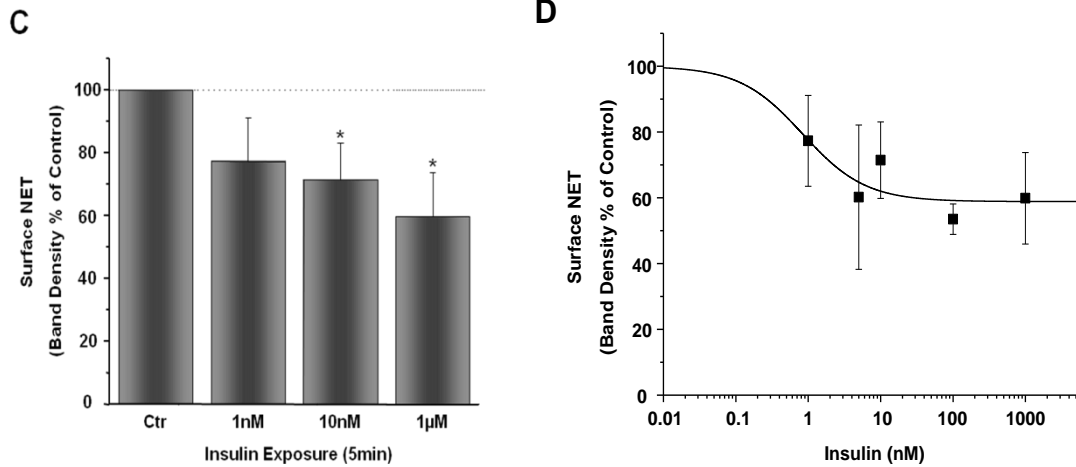
## RESULTS



**Figure 1:** A. Representative immunoblots for hNET recovered from biotinylated and total extract obtained from hNET cells treated as indicated. B. Quantification of immunoblots using Scion Image system. The density of the biotinylated samples were normalized to the density of the parallel total extract.

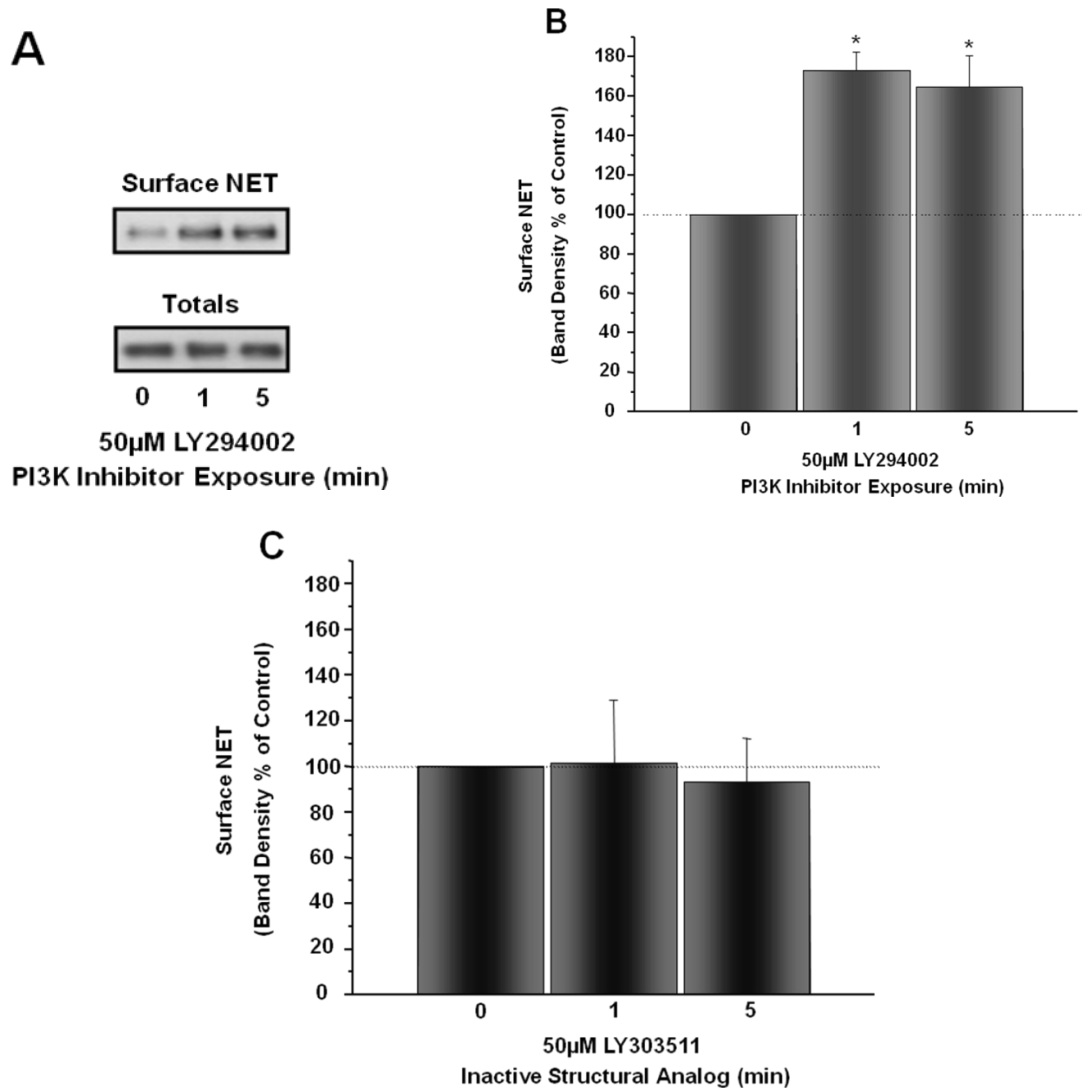
**Insulin Reduces NET Surface Expression.** Multiple studies have already demonstrated that insulin alters NET function, although the nature of this regulation has not been fully elucidated. To further characterize the hormonal regulation of hNET expression, we tested whether hNET cell surface expression can also be altered by the presence of insulin. Figure 1A shows immunoblots obtained from hNET cells treated with 1 μM insulin for the time periods of 0 minutes, 1 minute, and 5 minutes. As shown in Figure 1B, the quantification of the immunoblots, NET surface expression was decreased for both time exposures of 1 minute and 5 minutes, with a reduction of nearly 60% for the time exposure of 5 minutes. Therefore insulin reduces NET surface expression.





**Figure 2.** C. Quantification of immunoblots from multiple insulin dosage exposures. Immunoblots were obtained from hNET cells treated with vehicle (CTR), 1nM insulin, 10nM insulin, or 1μM insulin. D. Dose Response Curve

**Insulin Reduction in NET Surface Expression is Dose Dependent.** After demonstrating that insulin decreases NET surface expression at higher concentrations, we decided to examine more physiological concentrations. Figure 2C shows the quantification of immunoblots from different insulin concentration exposures. Immunoblots were obtained from hNET cells treated with vehicle (CTR), 1nM insulin, 10nM insulin, or 1μM insulin. As shown in Figure 2C and Figure 2D, the dose response chart curve, as the dose of insulin increases, the reduction in NET surface expression also increases.

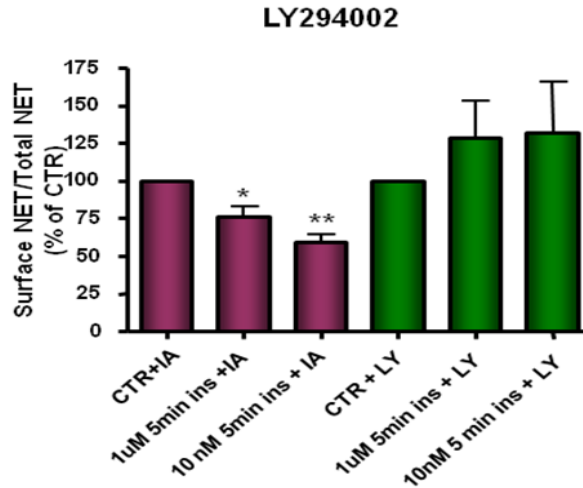


**Figure 3.** A. Representative immunoblots for hNET proteins recovered from biotinylated and total extracts obtained from hNET cells treated as indicated. B. Quantification of the immunoblots using Scion Image system. The density of the biotinylated samples was normalized to the density of the parallel total extract. C. Quantification of immunoblots using Scion Image system. Immunoblots were obtained from hNET cells treated with 50 µM LY303511, an inactive structural analog of LY294002, for time exposures indicated.

**PI3K Inhibition Enhances NET Surface Levels.** After providing evidence that insulin alters NET surface expression, we sought to determine which components of the insulin signaling pathway might play a role in the regulation of NET surface levels. Two well characterized and well known components of the insulin signaling pathway are PI3K

and Akt. Insulin receptors and insulin-like growth factor (IGF1-2) receptors function as receptor tyrosine kinases, which stimulate phosphatidylinositol 3-kinase (PI3K) signaling (C. Taha, A. Kilp, 1999). Through a series of phosphorylation events, PI3K activates another vital component of the insulin signaling pathway, protein kinase B (Akt). In these experiments, we sought to explore the role of PI3K in insulin's regulation of NET surface expression. Figure 3A shows immunoblots obtained from hNET cells were treated with 50  $\mu$ M LY294002, an inhibitor of PI3K, for time periods of 0 minutes, 1 minute, and 5 minutes. As shown in Figure 3B, the time exposures of 1 minute and 5 minutes show significant enhancement of NET surface levels in comparison to the time exposure of 0 minutes, with an increase of nearly 70% for the time exposure of 1 minute.

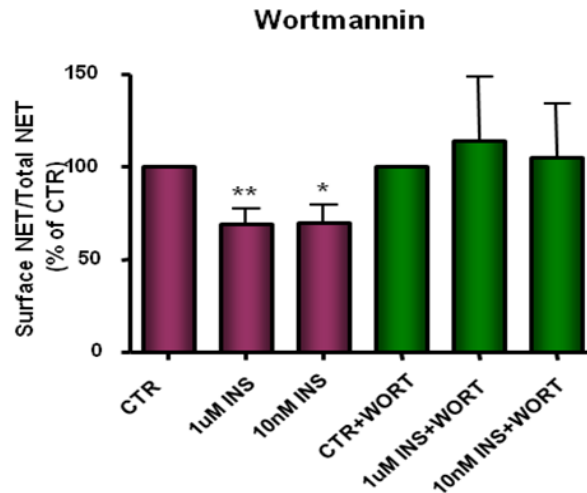
**Increases in NET Surface Levels are due Specifically to PI3K Inhibition.** Two inhibitors of PI3K are known, wortmannin and LY294002. In these experiments, we chose to use LY294002 because of its less harsh and slightly less potency in comparison to wortmannin. The results from the PI3K inhibition experiments showed that NET surface levels were enhanced when the inhibitor, LY294002, was applied. However, while we used the drug to inhibit PI3K, the results may have been due to the drug rather than the inhibition. Therefore, we sought to determine whether the enhancement in NET surface levels were due to strictly PI3K inhibition or non-specific actions of the drug. Cells were treated with 50  $\mu$ M LY303511, an inactive structural analog of LY294002, for time exposures of 0 minutes, 1 minute, and 5 minutes. As shown in Figure 3C, NET surface levels remained relatively the same. These data therefore indicate that the enhancement in NET surface levels is due specifically to PI3K inhibition, rather than non-specific actions of the drug.



**Figure 4.** Quantification of immunoblots using Scion Image system. Immunoblots were obtained from hNET cells treated as listed in figure. IA= structural inactive analog of LY294002, LY303511.

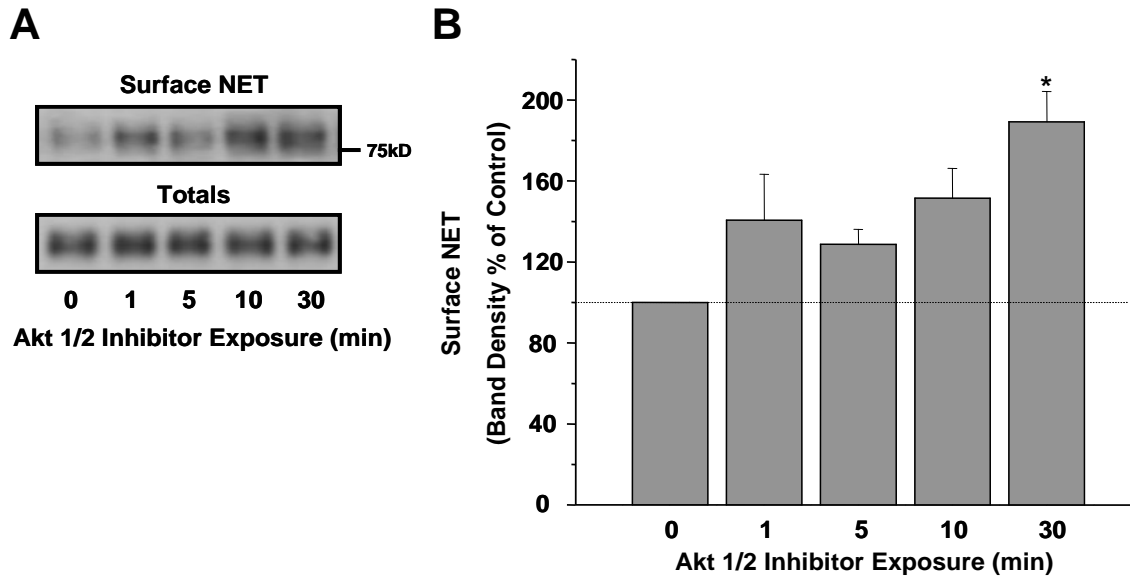
**LY294002 inhibits insulin-induced NET trafficking.** After providing evidence that LY294002 inhibits NET surface expression alone, we sought to determine if the PI3K inhibitor could also inhibit insulin-induced trafficking of NET. Insulin stimulation of CHO cells stably transfected with hNET was shown to cause a decrease in NET surface expression. If insulin regulation of NET requires PI3K, then insulin stimulation of the cells in the presence of the PI3K inhibitor should cause either no change in NET surface levels or even an increase in NET surface levels. CHO cells were incubated with LY294002 for one hour. Then cells were treated with insulin for five minutes in the presence of the inhibitor. The cells were then biotinylated and cell surface expression of NET was determined. As shown in Figure 5, control cells (those that were incubated with the structural inactive analog LY303511 rather than LY294002) were subject to the same insulin effect as previously shown. However, those cells that were incubated with LY294002 do not display decreased NET surface levels. In fact they show increased NET

surface levels. This further establishes the necessity of PI3K in insulin's regulation of NET.



**Figure 5.** Quantification of immunoblots using Scion Image system. Immunoblots were obtained from hNET cells treated as listed in figure.

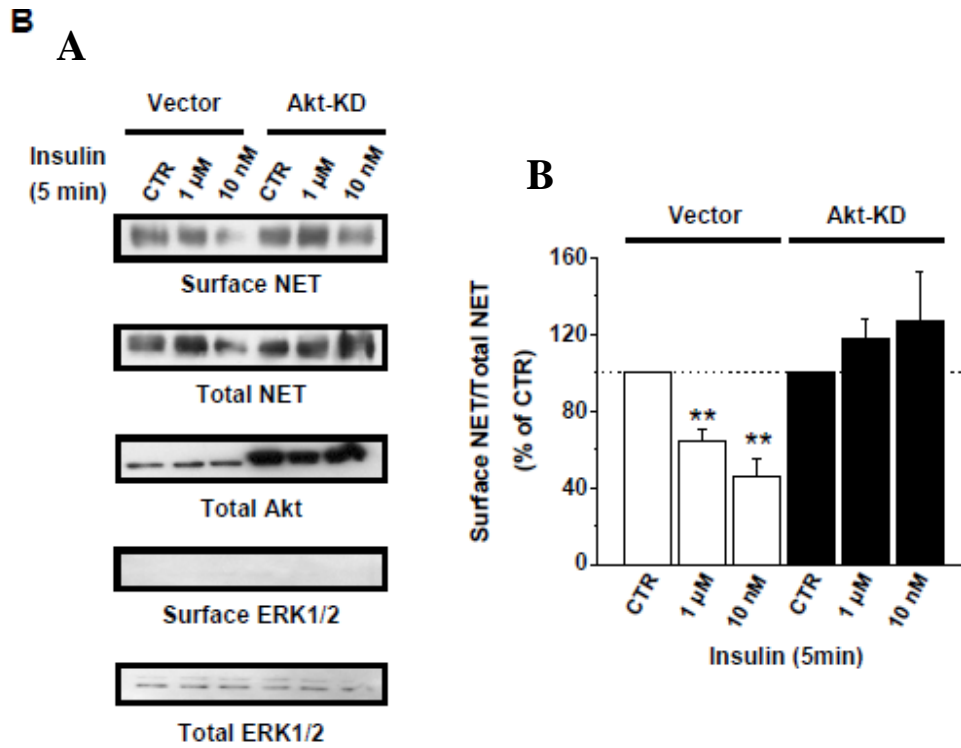
**Wortmannin inhibits insulin-induced NET trafficking.** Wortmannin is another inhibitor of PI3K. It is more potent and harsher of a pharmacological agent than LY294002. In order to further establish the necessity of PI3K in insulin's regulation of NET, the same experiment as previously described in relation to Figure 5 was performed, replacing LY294002 with wortmannin. The control cells show a clear downregulation in response to insulin stimulation. As shown in the figure, cells treated with wortmannin showed increased levels of surface NET, just as cells treated with LY294002 did. This once again illustrates the necessity of PI3K in the downregulation of NET by insulin through pharmacological inhibition.



**Figure 6.** A. Representative immunoblots for hNET proteins recovered from biotinylated and total extract obtained from hNET cells treated as indicated. B. Quantification of immunoblots using Scion Image system.

**AKT 1/2 Inhibition Enhances NET Surface Levels.** In the insulin signaling pathway, PI3K is necessary for the activation of protein kinase B, Akt. We sought to elucidate the role of Akt in insulin's regulation of NET surface expression. The Akt 1/2 inhibitor is an allosteric inhibitor that requires the pleckstrin homology (PH) domain of Akt to inhibit phosphorylation and activation of the kinase. While it requires this domain for inhibition, binding of the inhibitor to Akt requires the whole protein since *in vitro* assays show the PH domain alone is insufficient for binding. Furthermore, the inhibitor reversibly inhibits both the activation and activity of Akt and is highly specific for Akt compared to other similar kinases such as PKA, PKC, and SGK. Figure 7A shows immunoblots attained from hNET cells treated with an inhibitor of both Akt isoforms 1 and 2 for time exposures of 0 minutes, 1 minute, 5 minutes, 10 minutes, and 30 minutes.

As shown in Figure 7B, the inhibition resulted in enhanced levels of NET surface expression, with a nearly 80% increase for time exposure of 30 minutes.



**Figure 7.** A. Representative immunoblots for hNET proteins recovered from biotinylated and total extract obtained from hNET cells treated as indicated. B. Quantification of immunoblots using Scion Image system.

After establishing the pharmacological blockage, we sought to further characterize this regulation by using genetic inhibition. Here we also utilize a “kinase-dead” dominant negative mutant (K179R) construct of Akt (Akt-KD) to show that Akt activity is required for insulin induced regulation of the transporter (Garcia et al., 2005). This means that the transfected Akt is present but however cannot phosphorylate or be active in any way. Consistent with our previous results, transient transfection of hNET cells with the Akt-KD construct 48 hours prior to insulin application abolishes insulin stimulated trafficking of

NET away from the plasma membrane. This reiterates insulin's necessity of Akt in its regulation of NET.

### **CONCLUSION**

The results of the experimentation, for the first time, show that insulin, in a time and dose dependent manner, decreases NET cell-surface expression, thereby decreasing NET activity. Inhibition of PI3K, a central component of insulin signaling, resulted in enhanced levels of NET surface levels. Inhibition of insulin-induced trafficking with a PI3K inhibitor yielded the same results. The same results were attained from inhibition of protein kinase B (Akt), which requires PI3K for activation. Genetic inhibition of Akt resulted in enhanced levels of NET surface levels as well. By way of these experimental results, we conclude that insulin requires the activation of both components to decrease NET cell-surface expression. The compilation of these data suggest that states of hypoinsulinemia and insulin resistance, such as diabetes or obesity, may in fact alter NET surface expression and thereby NET function (i.e. NE uptake). Future studies will seek to elucidate the extent (i.e. whether important in both CNS and PNS) of the effects of PI3K and Akt in insulin regulation of NET and explore the physiological and behavioral consequences of specifically abolishing insulin receptor mediated insulin signaling in NET containing neurons.

### **DISCUSSION**

Prior studies imply a role for insulin in the regulation of NET. For example, insulin decreased NE uptake in a neuronal cell line, fetal brain synaptosomes, and in rat brain slices (Boyd et al., 1985, 1986; Figlewicz et al., 1993). Since these early studies, however, the nature of this regulation has remained elusive. Our data demonstrate that insulin



regulates NET activity by altering NET surface expression. Insulin application to hNET cells resulted in a striking time and dose dependent decrease in hNET surface levels. These results, for the first time, explain the role of insulin regulation of NET activity. Insulin regulates cell-surface NET levels and thereby levels of NE uptake, which explains the decrease in NE uptake seen in previous studies.

While some progress has been made, the signaling pathways involved in hormonal (e.g. insulin) regulation of neurotransmitter activity and cell-surface expression are not yet clearly understood. The downstream effects of insulin include the activation of PI3K, which is required for the activation of Akt, another key component of the insulin signaling pathway. In order to elucidate which components of the insulin signaling pathway are necessary for insulin regulation of NET, we began initially with the exploration of the role of PI3K. LY294002, a pharmacological inhibitor of PI3K, enhanced hNET surface expression. The inhibition of any component of the insulin signaling pathway important for the downregulation of NET should result in the enhancement of NET surface levels. Therefore the inhibition of PI3K demonstrates that PI3K plays an essential role in insulin regulation of NET. LY303511, an inactive structural analog of LY294002, did not stimulate any change in hNET surface expression, proving that the enhancement in hNET surface levels was due solely to the inhibition of PI3K. LY294002 also inhibited insulin-induced downregulation of NET. Therefore, by inverse, the data suggest that PI3K, a component of the insulin signaling pathway, is capable of regulating NET surface expression.

Akt is another central component in insulin and growth factor signaling and is activated as a result of PI3K activity. Three isoforms of Akt have been identified (Hanada

et al., 2004). All three Akt isoforms contain a pleckstrin homology domain that interacts with membrane lipid products of PI3K, and it is this interaction that is required for Akt activation (Hanada et al., 2004). Because of this specific interaction, we explored the role of Akt in insulin regulation of NET. In mouse tissue, both Akt1 and Akt2 isoforms are ubiquitously expressed, whilst Akt3 is more highly expressed in the brain and testis (Hanada et al., 2004). In hNET cells, we explored the role of Akt1 and Akt2 in insulin regulation of hNET cell-surface expression. Inhibition of Akt 1/2 showed enhanced hNET surface levels.

While evidence has been provided that insulin decreases NE uptake and now NET surface expression, insulin induces an increase in dopamine transporter (DAT) surface expression and function. Upon further exploration of the insulin signaling pathway, PI3K has been shown to be essential for insulin modulation of hDAT function in striatal synaptosomes and hDAT cells (Carvelli et al., 2002). PI3K inhibitor LY294002 blocked the insulin-induced increase in DA uptake (Carvelli et al., 2002). To the contrary, we provided evidence that suggests PI3K is involved in insulin regulation of NET, decreasing NET surface levels and thereby NET function.

We show that acute insulin treatment, within the physiological range, induces a decrease in NET surface expression, a result that has since been confirmed by new studies in the lab in mouse hippocampal slices and through the use of diabetic mouse models. Thus, these data suggest that states of hypoinsulinemia and insulin resistance, such as diabetes and obesity, may alter NET surface expression and therefore global noradrenergic tone in both the brain and the peripheral nervous system. Disruption of noradrenergic function in the brain has been linked to mood disorders such as depression, thus aberrant

insulin regulation of NET has the capacity to impact mental health. Therefore, these data suggest that insulin regulation of NET may provide a plausible link for insulin-related disorders such as diabetes and obesity to mood disorders such as depression. In addition, the data has shown that both Akt and PI3K may be involved in regulated global noradrenergic tone and thus overall mental and nervous system health.

### **ACKNOWLEDGEMENTS**

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# **Effects of Physical Stress-Induced Sleep Deprivation on Energy Stores in *Drosophila melanogaster***

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## **Abstract**

Research studies in humans have implicated that chronic sleep deprivation may have negative effects on metabolism, specifically in energy and fat storage. Sleep deprivation alone, however, has been shown to not have such adverse effects on metabolism and other factors, such as stress, must also be considered. Stress and energy storage pathways in *Drosophila melanogaster* are evolutionarily conserved through mammals, proving *Drosophila* stress responses to be a novel mechanism of measuring the adverse effects of stress and sleep deprivation. In this study flies were subjected to long-term sleep deprivation induced via physical stress from a mechanical stimulus. To assess changes in energy stores, total protein, whole-body triglycerides, and whole-body glycogen levels were measured and compared to those of the controls. No significant changes were observed in energy stores between the physically stressed groups and the controls. This finding is not consistent with other research suggesting adverse effects of stress and sleep deprivation on energy stores. Further experimentation will be required to determine the extent of recovery sleep and if compensation of energy stores occurs in *D. melanogaster* when subjected to long-term physical stress-induced sleep deprivation.

## **Introduction**

Recent human research studies have revealed widespread associations between

short sleep duration and heightened risk of obesity and type 2 diabetes (Cappuccio et al., 2008). Declared a global epidemic by the World Health Organization (WHO, 2010), simultaneous reduction in sleep time. A reduction of 2 hours of nightly sleep time has been observed in the last fifty years in the United States, according to the National Sleep Foundation (2009). In addition, positive correlations have been shown between increased body mass index (BMI) and short sleep times in populations of both males and females in varying age groups from children to adults. Amongst the obese, the odds of being a short-sleeper are increased 60-80% (Cappuccio et al., 2008).

It has been suggested that the activation of hormonal responses by short sleep may lead to obesity through elevated appetite and greater caloric intake. Reciprocal changes in the hormones leptin and ghrelin, both of which are involved in appetite regulation, are observed following rest deprivation. Reduced leptin and increased ghrelin may stimulate appetite such that weight-gain is favored (Taheri, Lin, Austin, Young, & Mignot, 2004).

*Drosophila* poses as a relevant model system for the study of sleep deprivation and its metabolic effects due to the many biological similarities shared with mammalian metabolic and sleep systems (Hendricks et al., 2000; Shaw et al. 2000). As in mammals, sleep in *Drosophila* is tightly regulated in both a homeostatic and circadian manner, and rebound is observed following rest deprivation (Hendricks et al. 2000). In this study, *Drosophila melanogaster* was used to measure the effect of sleep deprivation induced via physical stress on whole-body energy stores--protein, triglycerides, and glycogen. It has been shown in *Drosophila* that sleep deprivation alone when induced by a light stimulus does not have a significant impact on energy stores (Harbison & Sehgal, 2009). Therefore, flies were deprived of rest for six hours for six consecutive nights by constant

displacement and gentle rocking on a mechanical shaker, adding the factor of physical stress in this study. Because many of the basic metabolic functions found in *Drosophila* are evolutionarily conserved in mammals (Baker & Thummel, 2008; Shaw, Cirelli, Greenspan, & Tononi, 2000), measurement of energy stores is a relevant model of the effects of sleep deprivation on metabolism in human systems. In addition, triglycerides and glycogen have both been implicated in stress resistance in flies (Djawdan, Chippindale, Rose, & Bradley, 1998). Total protein, triglycerides, and glycogen levels in *Drosophila* subjected to sleep deprivation via physical stimulation were compared with that of the controls.

### **Methods and Materials**

Five wildtype fly lines (380, 399, 517, 774, and 820) originally from the *Drosophila* Genetic Reference Panel at North Carolina State University and supplied by the Bloomington Stock Center were used to assay the effects of sleep deprivation induced via physical stimulation on metabolism. Flies were reared and maintained on a yeast-sucrose-cornmeal standard medium at 25°C in a 12 hour light/dark cycle incubator. For the assays, adult flies 6-11 days old were collected and maintained at 20 flies per single-sex vial for the duration of the experiment until time of the assays. Four vials of flies per line were assayed: two vials per line were subjected to sleep deprivation via physical stimulation and two vials per line were established as the control. Food was available to flies *ad libitum*.

Long-term sleep deprivation was induced by subjecting flies to a mechanical stimulus constituting gentle rocking and constant displacement on a shaker device.

Shaking occurred alternating hours during the 12 hour dark period each night, totaling 6 hours of physical stimulation per night for 6 consecutive nights.

Flies were collected for homogenization following the sixth night of physical stimulation. Live weight was measured by weighing flies in groups of 10 to 0.1 mg using a Mettler Toledo AL54 balance. Whole flies were homogenized in a 0.01M  $\text{KH}_2\text{PO}_4$ , 1mM EDTA pH 7.4 buffer with 25  $\mu\text{L}$  of homogenizing buffer used per fly as described by Clark and Keith (1988). Homogenates were distributed and stored in Eppendorf standard micro test tubes at  $-8^\circ\text{C}$  as soon as possible. When needed for tests, homogenates were defrosted and subsequently used.

Assays involved the use of a NanoDrop® RD-1000 UV-Vis Spectrophotometer. Total protein was determined using Bradford's method with procedures as described in the Bradford Reagent technical bulletin from Sigma-Aldrich (#B 6916). BSA was used to develop the standardization curve. True serum triglycerides were measured using the Serum Triglyceride Determination Kit from Sigma-Aldrich (#TR0100) with volumes of reagents and samples decreased 10-fold to accommodate the small Eppendorf size. A 2-fold dilution scheme was used for construction of the Glycerol Reagent standardization curve. Total glycogen was measured using the PGO Enzymes assay from Sigma-Aldrich (#P 7119).

## **Results**

Sleep deprivation induced via physical stress did not have a significant impact on energy stores in *Drosophila melanogaster*. Concentrations of total protein, triglycerides, and glycogen amongst the five lines of flies were averaged and analyzed using Student's t-test, and a probability of 0.05 or less was considered statistically significant. No

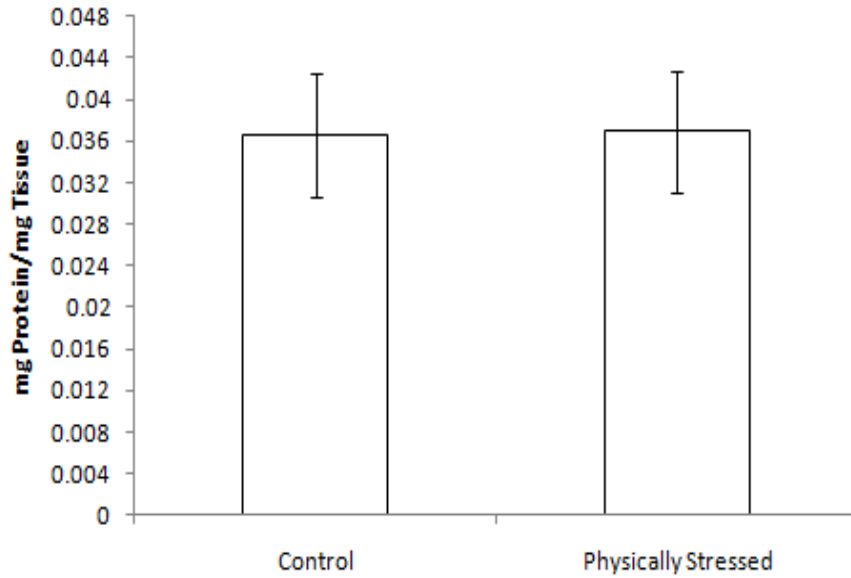


significant changes were observed in total protein, triglycerides, or glycogen between the control and physically stressed groups. (See Figure 1). While variation in regulation of energy stores may exist amongst the lines studied, further experimentation and analysis would be required to determine if any such differences are significant.

Mean total protein, triglycerides, and glycogen were further compared between males and females of both the physically stressed and control groups to elucidate differences in regulation of energy stores between genders. (See Figure 2). Females in both the control and physically stressed groups had slightly higher total protein than males, but this cannot, however, be considered statistically significant (Physically Stressed:  $t = -1.89$ ,  $p = 0.100$ ; Control:  $t = -1.72$ ,  $p = 0.123$ ). Significant differences in triglycerides between males and females of the physically stressed group were observed but not in the control group. Males were observed to have greater mg triglycerides per mg tissue than females in the same group ( $p < 0.05$ ). This same pattern was also reflected in comparing glycogen between males and females of the physically stressed and control groups. Significant differences were observed between males and females of the physically stressed group but not the control group. Males in the physically stressed group had greater mg glycogen per mg tissue than females in the same group ( $p < 0.05$ ).

Figure 1 (A & B) Mean total protein. No differences in mean total protein were observed between the control and physically stressed groups in *Drosophila melanogaster* (A. Females:  $t = -0.044$ ,  $p = 0.967$ ; B. Males:  $t = 0.054$ ,  $p = 0.958$ )

**A. Females: Mean Total Protein**



**B. Males: Mean Total Protein**

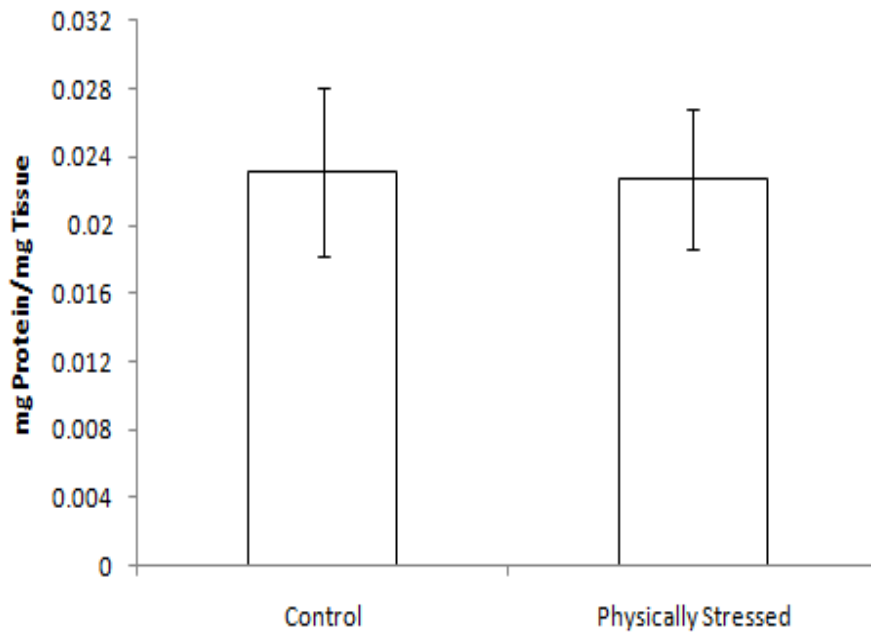
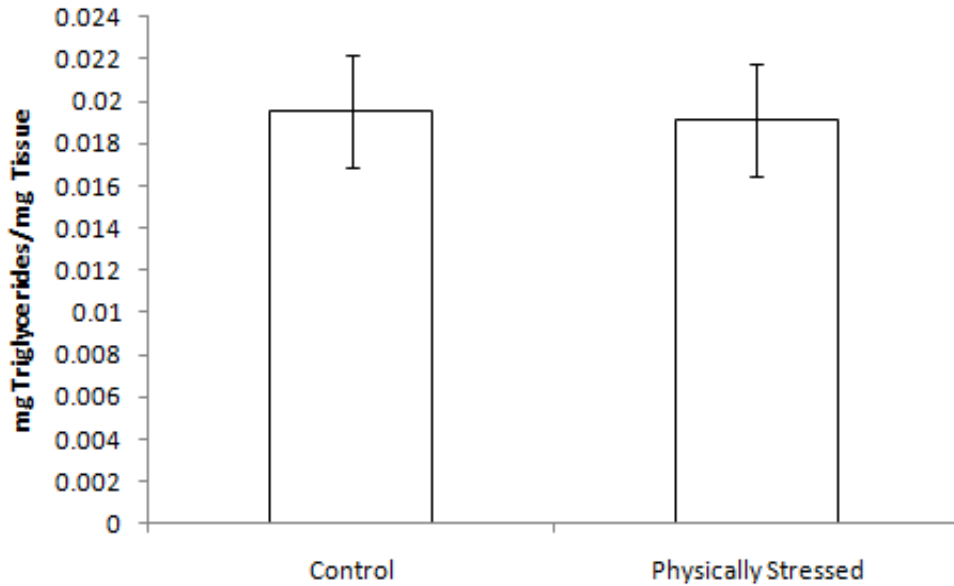


Figure 1 (C & D) Mean Triglycerides: No differences in mean triglycerides were observed between the control and physically stressed groups in *Drosophila melanogaster* (C. Females:  $t = 0.165$ ,  $p = 0.877$ ; D. Males:  $t = 0.09$ ,  $p = 0.930$ )

### C. Females: Mean Triglycerides



### D. Males: Mean Triglycerides

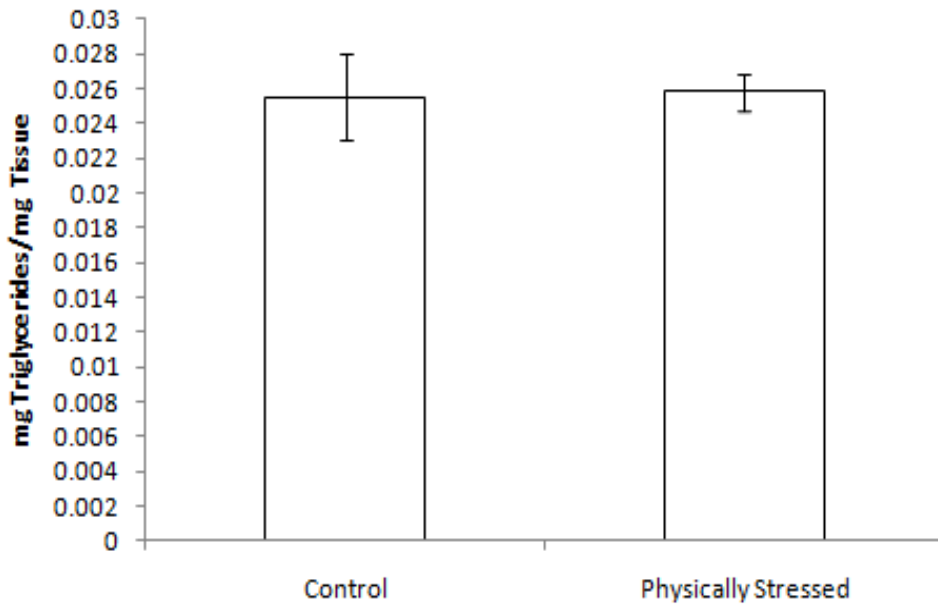
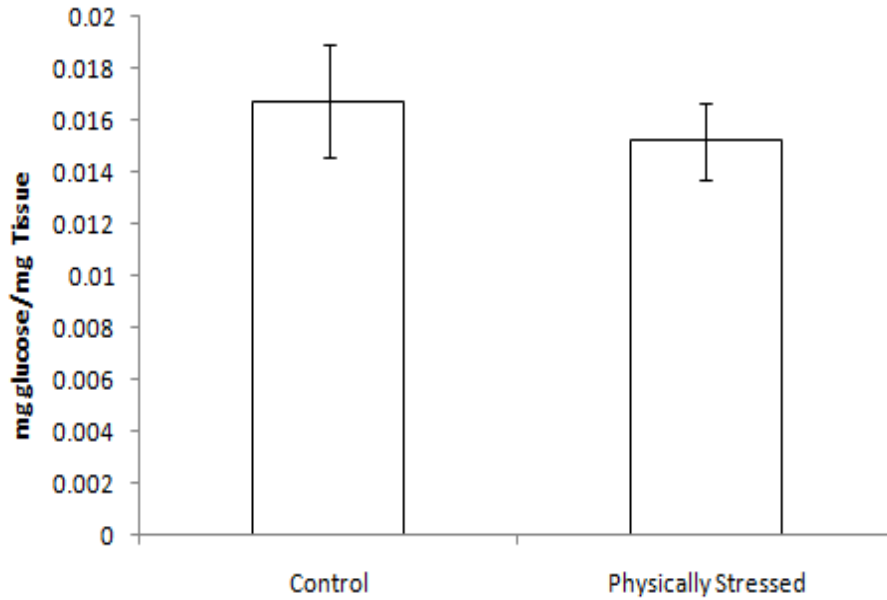


Figure 1 (E & F) Mean Glycogen: No differences in mean glycogen were observed between the control and physically stressed groups in *Drosophila melanogaster* (E. Females:  $t = 0.247$ ,  $p = 0.682$ ; F. Males:  $t = 0.40$ ,  $p = 0.708$ )

### E. Females: Mean Glycogen



### F. Males: Mean Glycogen

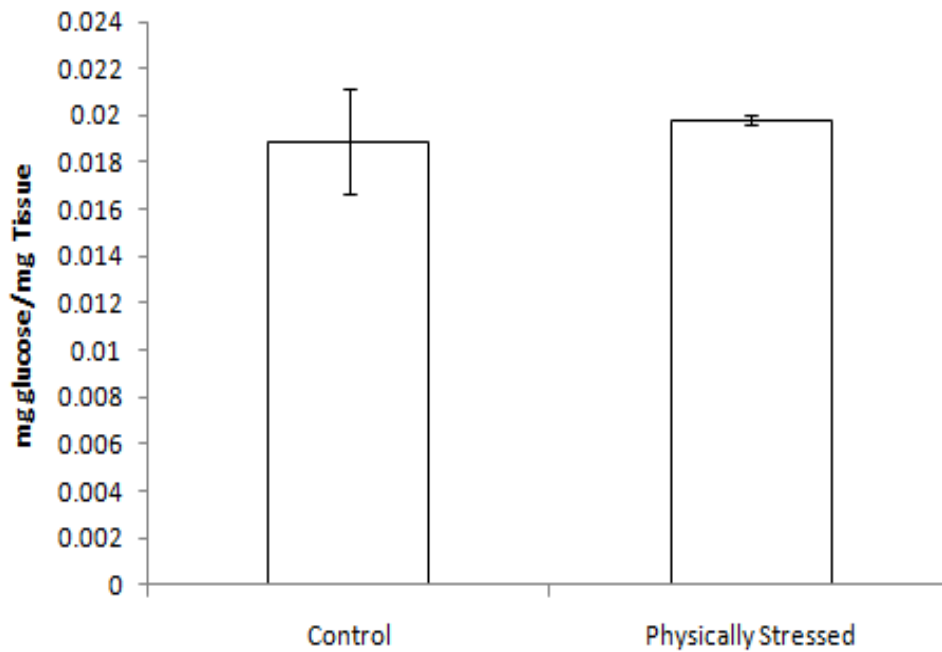
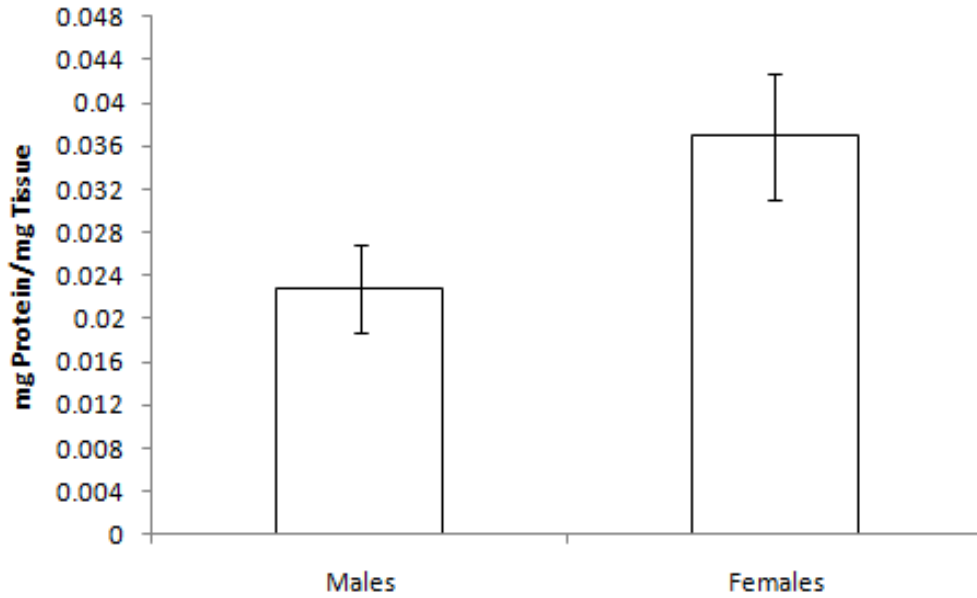


Figure 2 (A & B) Comparison of total protein in males and females of the physically stressed and control groups. No statistically significant differences were observed in total protein. (A. Physically Stressed:  $t = -1.89, p = 0.100$ ; B. Control:  $t = -1.72, p = 0.123$ )

**A. Mean Total Protein: Physically Stressed**



**B. Mean Total Protein: Control**

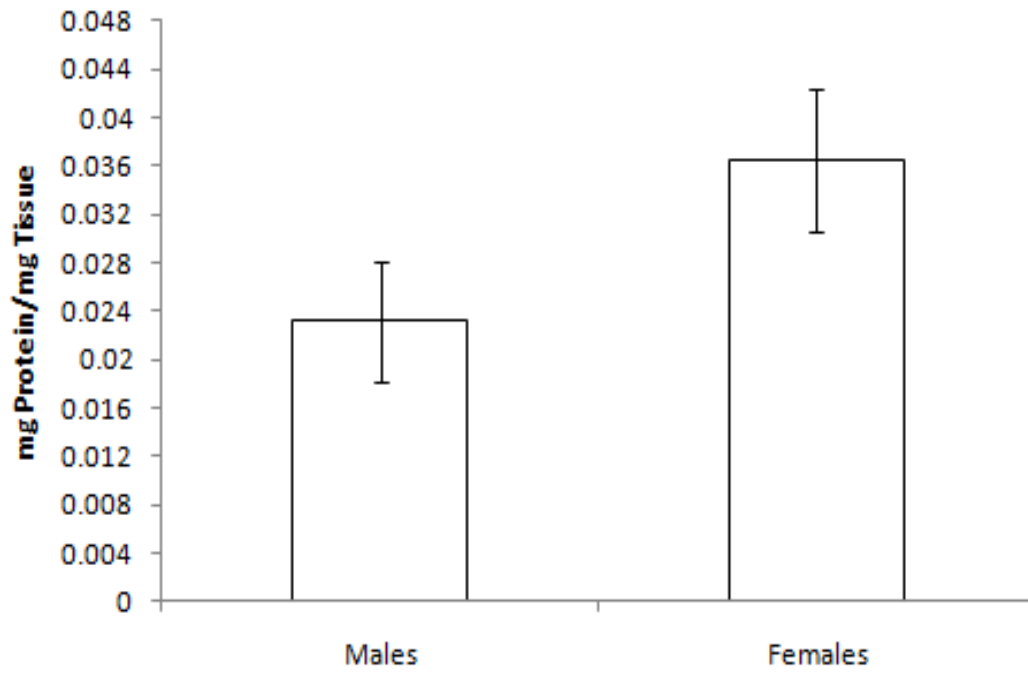
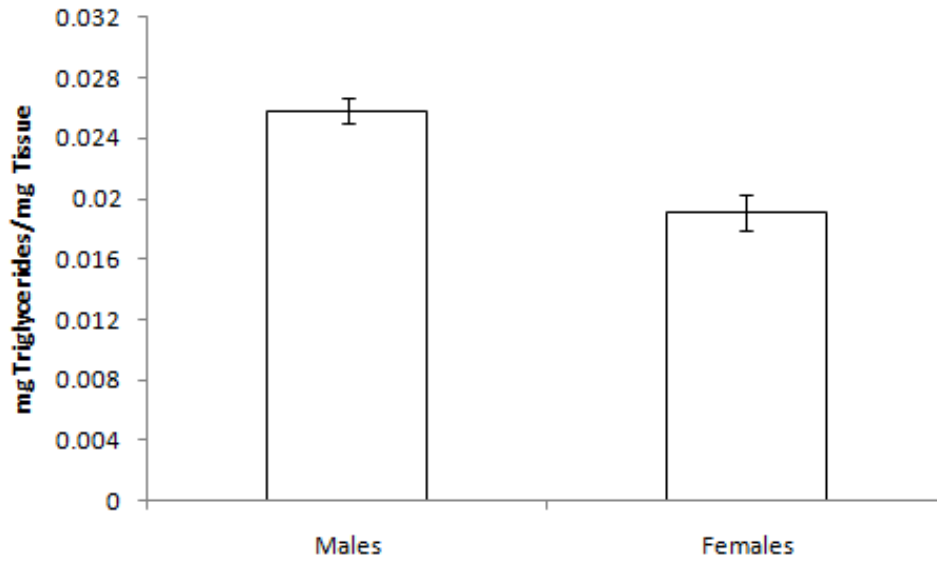


Figure 2 (C & D) Significant differences in both triglycerides and glycogen were observed between males and females of the physically stressed groups (C. Physically Stressed:  $t = 4.37, p = 0.05$ ; D. Control:  $t = 1.46, p = 0.181$ )

### C. Mean Triglycerides: Physically Stressed



### D. Mean Triglycerides: Control

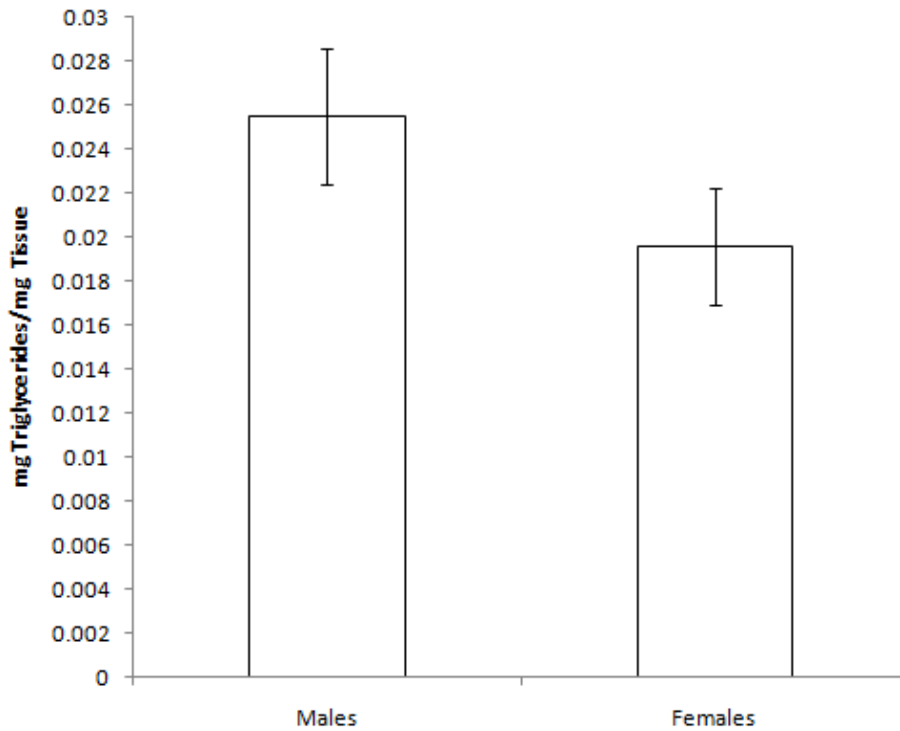
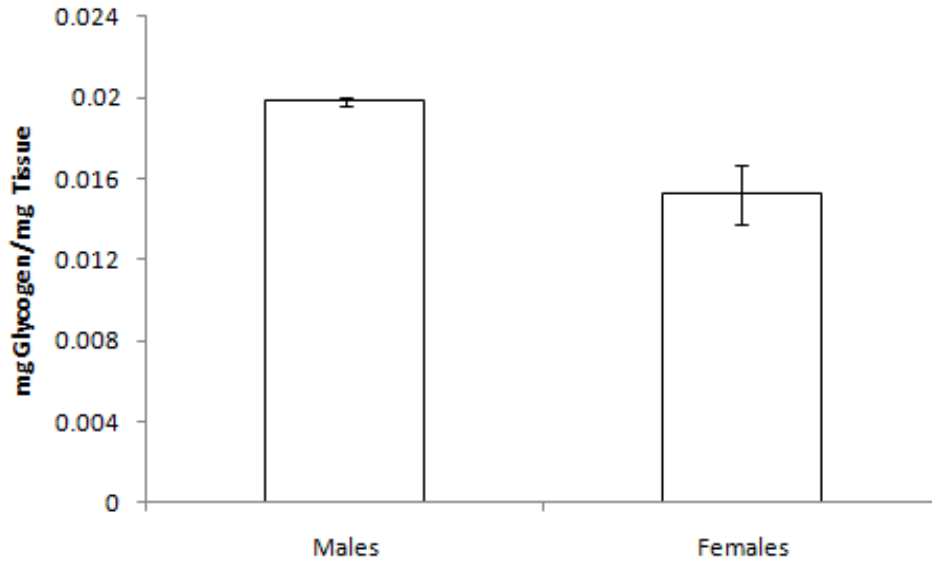
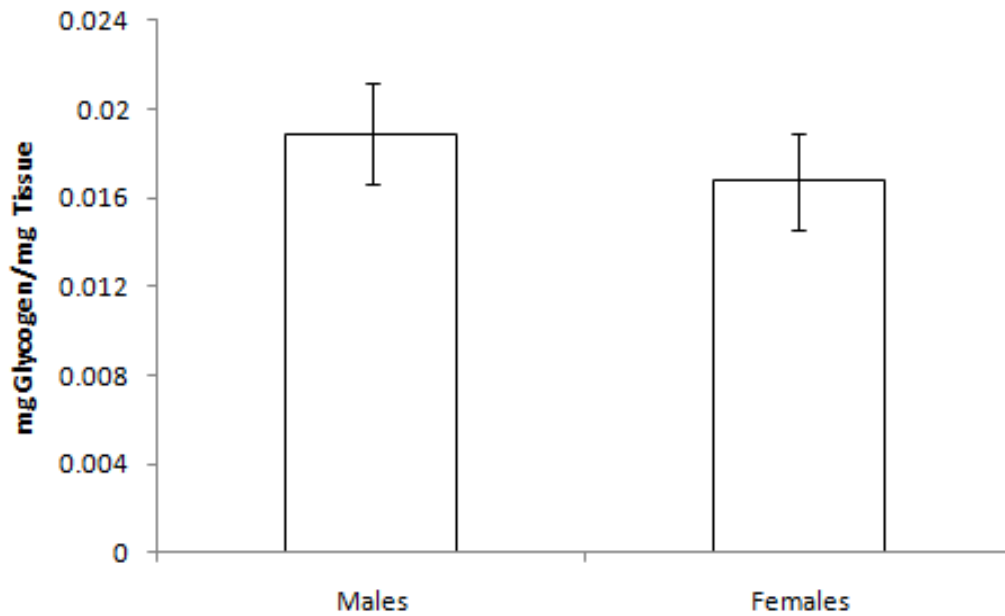


Figure 2 (E & F) Significant differences in glycogen were observed between males and females of the physically stressed groups (E. Physically Stressed:  $t = 3.04, p = 0.05$ ), but not the control groups (F.  $t = 0.86, p = 0.515$ )

### E. Mean Glycogen: Physically Stressed



### F. Mean Glycogen: Control



## **Discussion**

The results of this experiment show no significant changes in energy stores in *Drosophila melanogaster* when subjected to sleep deprivation induced via physical stress. These results are inconsistent with previous studies suggesting that sleep deprivation and stress have a significant impact on energy stores and metabolism. While previous studies have observed stress response pathways, such as the c-Jun N-terminal kinase conserved in flies, that influence triglyceride and fat storage (Stronach & Perrimon, 1999), this experiment does not support such research. It is possible that *Drosophila*, as suggested by other studies, compensate for sleep lost during periods of physical stress through recovery sleep during the day. Recovery sleep has, however, been shown to only partly compensate for lost sleep (Youngsoo, Laposky, Bergmann, & Turek, 2007), and further experimentation and analysis of *Drosophila* sleep patterns when subjected to physical stress will be required to determine the extent of recovery sleep. The other possibility exists that *Drosophila* eating patterns are altered when subjected to shaking in the mechanical device inducing physical stress. Flies may compensate for sleep lost through regulation of energy stores, but detection of this may have been inhibited by the possibility that eating patterns might be disrupted due to constant displacement of flies when subjected to shaking in the mechanical device. Further experimentation will be required to determine if eating patterns are at all altered in flies when subjected to the mechanical stimulus and if compensation in energy stores can be detected.

## **Acknowledgements**

Special thanks must be given to Dr. Lev Yampolsky of the East Tennessee State



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# **Doped Cadmium Sulfide as a Photocatalyst for Water Decomposition – A Second Year Study: Reaction Kinetics**

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## **Abstract**

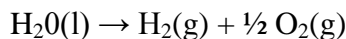
Cadmium sulfide doped with lead sulfide can be used as a photocatalyst for the decomposition of water. The research here presented was conducted in order to determine the best method for preparation of the catalyst, the rate law, and the activation energy for the decomposition. Cadmium sulfide containing 10 mole percent of lead sulfide as a co-precipitate and vacuum dried at 150°C was effective at 20°C with a rate of  $1.6 \times 10^{-7}$  mole·s<sup>-1</sup> of H<sub>2</sub>. However, as the temperature of the reaction mixture increased, the rate decreased to  $0.8 \times 10^{-7}$  mole·s<sup>-1</sup> at 40°C, and  $0.3 \times 10^{-7}$  mole·s<sup>-1</sup> at 60°C. From these data, by using the method of initial rates, the reaction order was calculated to be “0”, and the rate law became the rates at these temperatures. The activation energy was further calculated using the Arrhenius equation to be 26.5 kJ·mole<sup>-1</sup> from 20°C to 40°C and 43.7 kJ·mole<sup>-1</sup> from 40°C to 60°C. Since this photocatalyst does indeed decompose water effectively at room temperature and atmospheric pressure, the hydrogen can be used from this reaction as an alternative fuel source to decrease the amount of greenhouse gases that are being emitted into the atmosphere.

## **Introduction**

Considering the current interest in alternative energy sources (especially those that do not contribute to greenhouse gases), hydrogen production would be an ideal alternate

source. Furthermore, photocatalysis of water decomposition has been reported as a potential method for hydrogen production (1, 2, 14).

Photocatalysis is the acceleration of a photoreaction in the presence of an appropriate catalyst. When a photocatalyst is exposed to light of the proper wavelength range in the presence of water, the water decomposes into hydrogen and oxygen gases.



Photocatalytic activity has been demonstrated with several common compounds such as titanium dioxide. In fact the uses of photocatalysts have been as diverse as:

1. Fog-proof and self-cleaning glass
2. A bactericide, a virucide, and a fungicide
3. Anti-soiling, self-cleaning ovens
4. Deodorizing, air purification
5. Water treatment, water purification

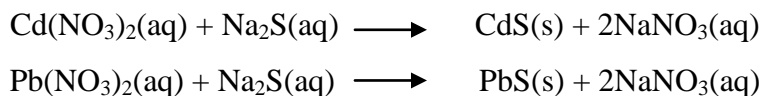
The mechanism of action for a photocatalyst is to establish a photo-excitation state in a manner similar to that of an n-type semiconductor in which a doping material provides extra valence electrons (9, 10, 16). For  $\text{TiO}_2$ , the wavelength of light needed for this photo-excitation is in the order of 390 nm (16).

Cadmium sulfide (CdS) has shown promising photocatalytic activity in the decomposition of water (11, 12, 13). CdS doped with a small amount of molybdenum disulfide ( $\text{MoS}_2$ ) serves as an efficient photocatalyst for the water-splitting reaction under visible white light. By using a wet chemical procedure to load CdS with just 0.2% by weight of  $\text{MoS}_2$ , the activity was increased by a factor of 36 (1). Mechanical mixtures of

MoS<sub>2</sub> and CdS, however, were not effective for generating hydrogen from water. Both substances must be co-precipitated.

Hydrogen is viewed as a pollution-free, clean energy source that could be substituted for existing fossil fuels. Most, if not all, conventional methods for hydrogen production are not economically favorable because immense heat, electrical energy, or expensive catalysts are needed to produce it from water. Prior research using photocatalysts to produce hydrogen and oxygen from water has generated only small quantities.

Cadmium sulfide and lead sulfide are effectively co-precipitated by reaction in an aqueous solution of the metal nitrates with sodium sulfide.



The two insoluble sulfides are filtered, washed, and vacuum dried to produce the photocatalyst. This catalyst is activated by mixing it with a solution of ferric chloride and sodium sulfite.

The previous research was done in our laboratory in an effort to develop a procedure for preparation of an effective catalyst and to test its activity at different temperatures. This second-year study was undertaken to further investigate a best practice for catalyst preparation and to calculate the rate law and activation energy by using the method of initial rates and the Arrhenius equation.

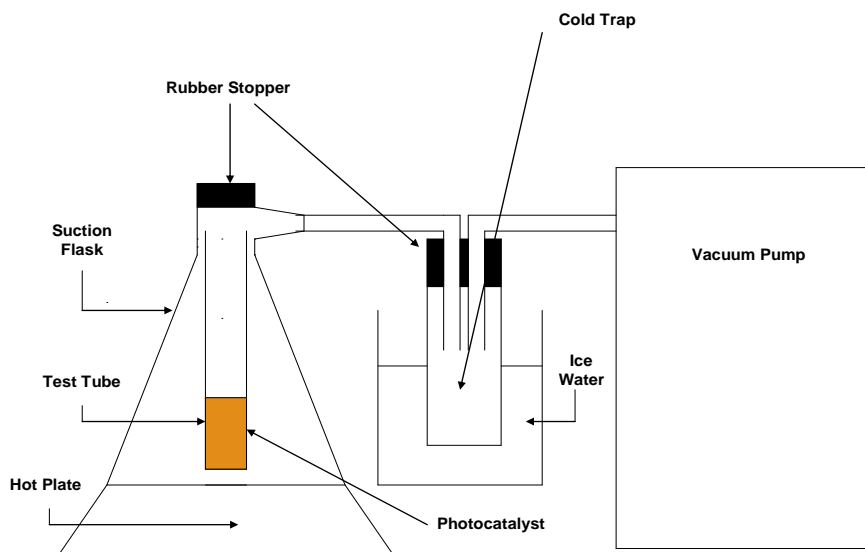
## Methods and Materials

### *Preparation of the photocatalyst:*

The procedure for preparing the photocatalyst (8) as modified from the prior research to decompose water is to dissolve 21.4 g of cadmium nitrate ( $\text{Cd}(\text{NO}_3)_2$ ) in 250 mL of DI water prepared with a Barnstead deionization system. 2.49 g of lead nitrate ( $\text{Pb}(\text{NO}_3)_2$ ) is then dissolved in the same solution. While stirring, 40 mL of sodium sulfide ( $\text{Na}_2\text{S}$ ) solution (2.0 M) is added to co-precipitate the metal sulfides.

The precipitate is filtered through a Buchner funnel and washed in the funnel with DI water. The wet precipitate is placed into a test tube. The test tube is then placed into a filtering flask, a vacuum is pulled, and the flask is heated on a hot plate as done in the previous research (Figure 1) for two to three hours at approximately  $150^\circ\text{C}$ .

### **Apparatus for Vacuum Drying the Photocatalyst**



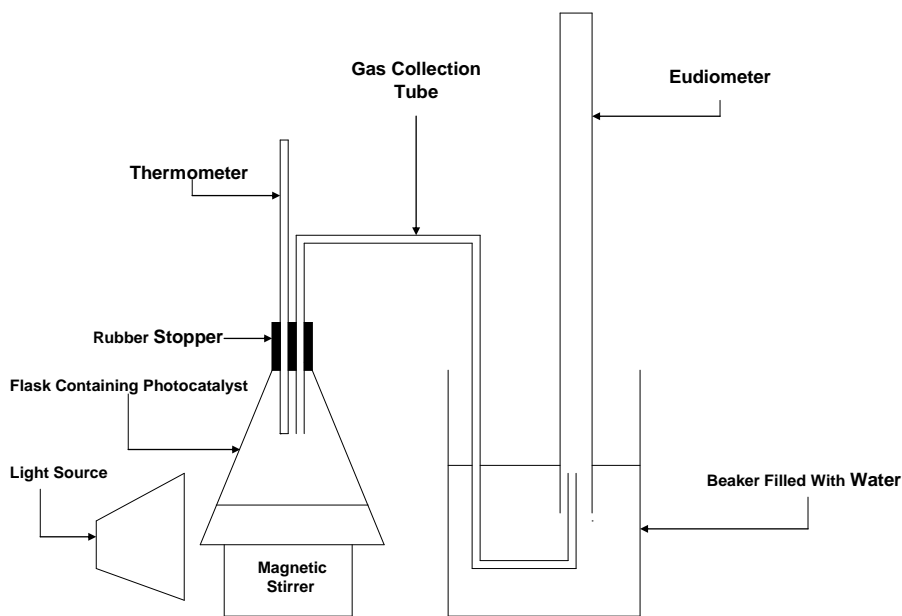
**Figure 1**

*Use of the photocatalyst for the decomposition of water:*

0.56 g of ferric chloride ( $\text{FeCl}_3$ ) is dissolved in 25 mL of DI water. The dry precipitate from the photocatalyst preparation is added to this solution to form a slurry. 7.1 g of sodium sulfite ( $\text{Na}_2\text{SO}_3$ ) and 25 mL of 2.0 M  $\text{Na}_2\text{S}$  solution are mixed and dissolved in another 75 mL of water. Both mixtures are then combined. While stirring with a magnetic stirrer, this combination is illuminated with a flood lamp rated at 1300 lumens. The generated gas from the water decomposition is collected in a eudiometer (for accurate volume measurement) over water as a function of time (Figure 2).

To calculate the rate of reaction for the decomposition, the temperature was brought to the desired level, and the gas was collected for thirty minutes.

### **Apparatus for Testing of CdS as a Photocatalyst**



**Figure 2**

*The rate law and the activation energy for the reaction:*

The volume of gas collected, along with temperature and pressure, was used to calculate the rate of the reaction in moles per second using the Ideal Gas Law,

$$PV = nRT$$

P = hydrogen pressure in atmospheres (water vapor pressure and oxygen pressure were subtracted from the total pressure per Dalton's Law of Partial Pressures,  $P_{H_2} = P_T - P_{O_2} - P_{H_2O}$ ), V = volume of hydrogen collected (hydrogen volume is two-thirds of the total), n = moles of hydrogen, R = 0.0821 L·atm·mole<sup>-1</sup>·K<sup>-1</sup>, and T = Kelvin temperature. Rate was then calculated by the following equation,

$$\text{Rate} = n/t$$

n = moles from the Ideal Gas Law, t = time in seconds.

For the rate law, Rate = k[H<sub>2</sub>O]<sup>x</sup>, the rate remained constant and the water concentration remained constant over the course of the reaction indicating a reaction order of x = 0. This is a typical result of a catalyzed reaction of this type. Therefore, the rate law becomes,

$$\text{Rate} = k.$$

The data were further used to calculate the activation energy (3, 4, 5, 6, 7, 8, 15) from the Arrhenius equation,

$$k = Ae^{-E_a/RT}$$

k = rate constant, A = the reaction frequency factor, E<sub>a</sub> = activation energy, R = 8.314 J·mole<sup>-1</sup>·K<sup>-1</sup>, and T = Kelvin temperature, which converted to

$$\ln k = -(E_a/R)1/T + \ln A.$$

ln k was plotted versus 1/T and the slope determined as -E<sub>a</sub>/R. ln A was determined from the y-intercept.



## Results and Discussion

CdS doped with PbS as co-precipitates using the formulation presented above and vacuum dried at 150°C was effective as a photocatalyst for water decomposition upon exposure to a flood lamp with a luminosity of 1300 lumens. As the temperature of the photocatalytic slurry was increased, the water decomposition decreased to the point that at 60°C the reaction was only about one-eighth that of 20°C.

At a slurry temperature of 20°C the reaction rate as indicated in Figure 3 was slightly greater than  $1.6 \times 10^{-7}$  mole·s<sup>-1</sup> of H<sub>2</sub>. This rate decreased to approximately  $0.8 \times 10^{-7}$  mole·s<sup>-1</sup> of H<sub>2</sub> at 40°C, and further decreased to  $0.3 \times 10^{-7}$  mole·s<sup>-1</sup> of H<sub>2</sub> at 60°C.

The rates allowed determination of the rate constant to be  $1.6 \times 10^{-7}$  at 20°C,  $0.8 \times 10^{-7}$  at 40°C, and  $0.3 \times 10^{-7}$  at 60°C with a reaction order of 0. Applying the Arrhenius equation to these data (Figure 4) further allowed the calculation of the activation energy, E<sub>a</sub>, to be 26.4 kJ·mole<sup>-1</sup> between 20°C and 40°C and 43.7 kJ·mole<sup>-1</sup> between 40°C and 60°C.

**Table 1**

<u>Temperature (°C)</u>	<u>Rate Law</u>	<u>ln k</u>	<u>1/T</u>
20	$1.6 \times 10^{-7}$	-15.6	$3.41 \times 10^{-3}$
40	$0.8 \times 10^{-7}$	-16.3	$3.19 \times 10^{-3}$
60	$0.3 \times 10^{-7}$	-17.3	$3.00 \times 10^{-3}$

Figure 3

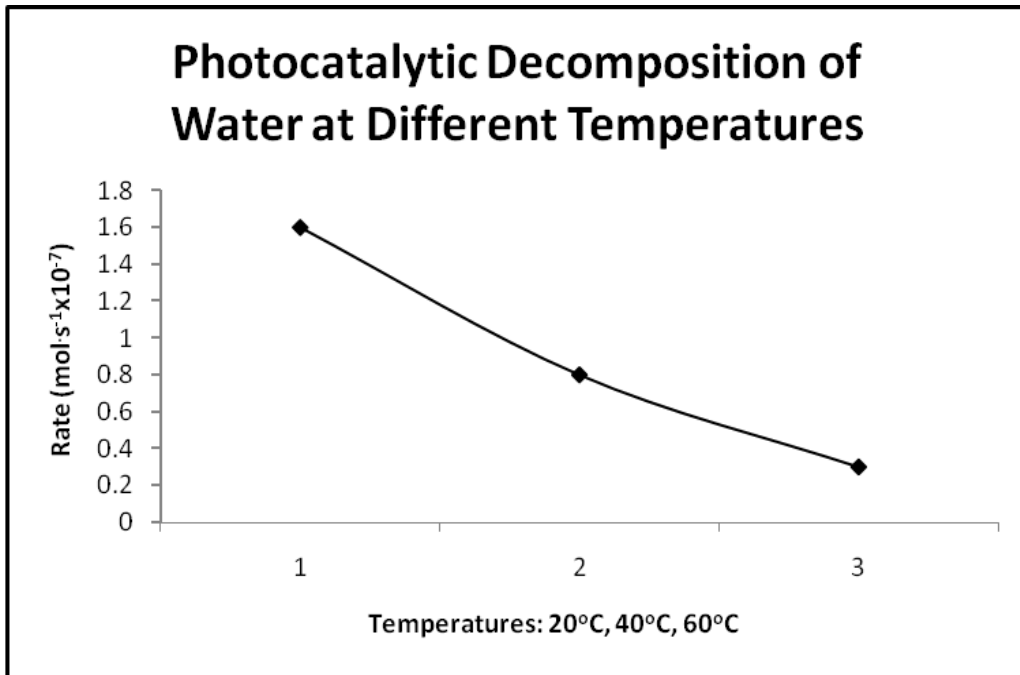
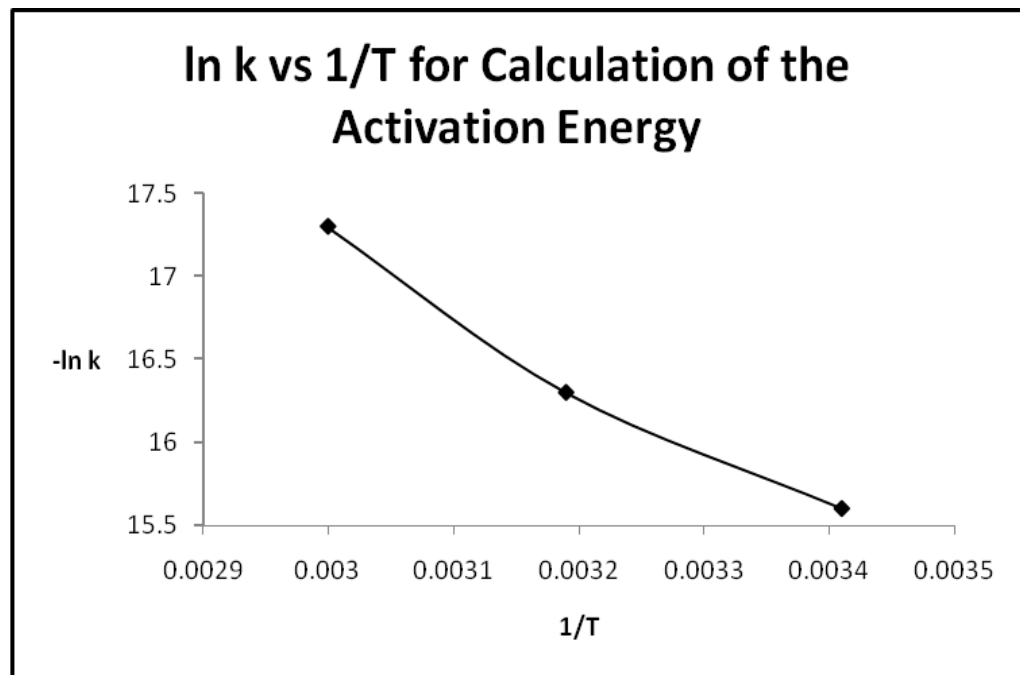


Figure 4



## **Conclusions**

Increasing the temperature of the reaction mixture decreased the rate of water decomposition from  $1.6 \times 10^{-7} \text{ mole}\cdot\text{s}^{-1}$  at  $20^\circ\text{C}$  to  $0.3 \times 10^{-7} \text{ mole}\cdot\text{s}^{-1}$  at  $60^\circ\text{C}$ . This caused a continuous change in activation energy,  $E_a$ , from  $26.4 \text{ kJ}\cdot\text{mole}^{-1}$  to  $43.7 \text{ kJ}\cdot\text{mole}^{-1}$ . Thus, lead sulfide doped cadmium sulfide has been shown here to be an effective photocatalyst for the decomposition of water at room temperature ( $\sim 20^\circ\text{C}$ ), atmospheric pressure, and in the presence of a reducing agent ( $\text{Na}_2\text{SO}_3$ ).

The procedure here presented potentially provides a simple, efficient, and inexpensive method for the production of hydrogen as an alternative fuel to aid in the decrease of greenhouse gases being emitted into the atmosphere.

## **Future Direction**

Scaling up the process to make it commercially feasible for hydrogen production would be the next step in this research.

## **Acknowledgements**

I would like to thank Mr. Ronnie Nixon who encouraged, informed, and assisted throughout my research. His help was very much appreciated, and I couldn't have done it without him. Thank you.

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# **Inhibition of Zebrafish Cyclooxygenase by Nonselective and COX-2 Selective Inhibitors**

Tim Clavin  
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## **Abstract**

Cyclooxygenase (COX) enzymes catalyze the conversion of arachidonic acid (AA) to prostaglandin H<sub>2</sub> (PGH<sub>2</sub>), the common biosynthetic precursor to prostaglandins and thromboxane (1). Two COX isoforms have been identified in mammals (COX-1 & COX-2) and three in zebrafish (COX-1, COX-2a, COX-2b). In mammals, COX-1 is widely expressed and is required for the production of prostaglandins involved in basic housekeeping functions throughout the body. COX-2 is primarily an inducible enzyme (constitutively expressed in the brain and kidneys) whose expression is activated in response to cytokines, mitogens, and endotoxins (2). COX has been shown to be important in zebrafish in the gastrulation stage of development (3). Little is known about inhibition of zebrafish COX by either nonselective or COX-2 selective inhibitors. Our studies examined inhibition of zebrafish COX by nonselective inhibitors (ibuprofen, indomethacin, flurbiprofen) and COX-2 selective inhibitors (celecoxib and rofecoxib). The nonselective inhibitors were able to inhibit both zCOX-1 and -2a similarly to mammalian COX. Celecoxib inhibited zCOX-1 with an IC<sub>50</sub> of 4.8 mM and zCOX-2a with an IC<sub>50</sub> of 0.45 mM. Rofecoxib displayed little inhibition of zCOX-1 but had an IC<sub>50</sub> of 0.45 mM against zCOX-2a. Val-434 and Val-523 in mammalian COX-2 are important residues for inhibition by COX-2 selective inhibitors. Our project also

examined the role of residues 523 and 434 in zCOX-1 and zCOX-2a. Results show that the Val-523 plays a major role in the binding of COX-2 selective inhibitors to zCOX-2a, while Val-434 plays a minor role for the same case. However, this is not true for zCOX-1. zCOX-1 also contains Val-523 but is poorly inhibited by COX-2 selective inhibitors. In mammalian COX isoforms, Val-523 is thought to be the primary residue that allows for design of COX-2 specific inhibitors. Since both zCOX-1 and zCOX-2a contain Val-523, the results of inhibition of zCOX-2a and zCOX-1 by celecoxib and rofecoxib point to other as yet undetermined amino acids that influence binding of COX-2 selective inhibitors. These amino acids could also potentially be important in inhibition of mammalian COX-2 and possibly exploited for future drug design.

### **Introduction**

Cyclooxygenase (COX) enzymes catalyze the conversion of arachidonic acid (AA) to prostaglandin H<sub>2</sub> (PGH<sub>2</sub>), the common biosynthetic precursor to other prostaglandins and thromboxane (Gierse, 1996). While numerous people depend on cyclooxygenase enzymes for pain relief regulation on a daily basis, little is known about the specific mechanisms behind their function. However, the commonality of COX inhibitors signifies an important point in coming to an educated conclusion about the specifics of cyclooxygenase enzymes. The main purpose of this research is to explore the specific mechanisms behind cyclooxygenase inhibition; it has been hypothesized that the valine located at residues 434 and 523 in zCOX-2a, and the isoleucine located at residue 434 in zCOX-1 play an important role in the binding of inhibitor to zCOX-2a.

*zCOX and hCOX Isoforms*

Two COX isoforms have been identified in mammals (COX-1 and COX-2) and three in zebrafish (COX-1, COX-2a, COX-2b). In mammals, the COX-1 enzyme is widely expressed and required for the production of prostaglandins involved in basic housekeeping functions throughout the body. On the other hand, COX-2 is primarily an inducible enzyme, constitutively expressed in the brain and kidneys. Its expression is activated mainly in response to cytokines, mitogens, and endotoxins (COX Protein, 2009). For zebrafish, cyclooxygenase enzymes have shown to play an important role in its gastrulation stage of development (Cha, 2006). In addition, mammals and zebrafish have been found to have similar amino acid sequences for the COX enzyme. This information establishes an evolutionary standpoint for the basis of working with zebrafish as a test subject.

#### *COX Inhibition*

Little is known about the role amino acid sequencing plays in the binding of nonselective and COX-2 selective inhibitors to zCOX-1 and 2a. Current drugs on the market act as both nonselective, and COX-2 selective inhibitors. These drugs belong to a class of larger drugs called Non-steroidal anti-inflammatory drugs (NSAIDs). Common nonselective inhibitors are available over the counter, including aspirin, ibuprofen, and naproxen. Most COX-2 selective inhibitors, however, are only available through a doctor's prescription, and include celecoxib (Celebrex) and rofecoxib (Vioxx). While COX-2 inhibition carries a positive, therapeutic effect, continued inhibition of COX-1 coincides with loss of beneficial prostaglandins in the gastrointestinal tract, leading to chronic GI problems such as ulcers and stomach cancer. Because of this, there is an issue in using nonselective NSAIDs over a prolonged period of time. On the contrary, COX-2



specific inhibitors have been known to cause cardiovascular problems (Prescott, 2002). In the past few years, Vioxx has been removed from the market due to its increased risk in heart attack in patients. More recently, Celebrex has been placed on the FDA watch-list due to possible cardiovascular adverse effects. Scientists have speculated that this risk of developed cardiovascular issues may be attributed to an increased level in thromboxane that occurs from attempting to maintain COX-1 levels with the use of COX-2 specific inhibitors.

#### *Cyclooxygenase Active Site Structure*

NSAIDs are competitive cyclooxygenase inhibitors, and the NSAID binding site corresponds to the cyclooxygenase active site. The active site of COX-2 is known to be approximately 20% larger, and have a slightly different shape than that of COX-1. This difference in size and shape is mainly attributed to three amino acid differences between COX-1 and 2: Ile523 to Val523 in the first shell of the active site, and Ile434 to Val434 and His513 to Arg513 in the surrounding second shell. The other major structural difference is the position of helix D, the last of the four helices of membrane binding domain (MBD). For COX-2, helix D is cantilevered upward to provide a larger opening in the MBD. These various differences in the active sites of COX-1 and COX-2 influence inhibitor binding and provide more substrate flexibility in the COX-2 site (Smith, 2000).

### **Materials and Methods**

*Cloning.* An EcoRI/XhoI digest of zCOX-2b/pDNR-LIB was performed. The 2.5 kb band from the gel (ran on this sample) was extracted, purified, and ligated to EcoRI/XhoI-digested pENTR4 vector. The zCOX-2b was then transferred from the

pENTR4 vector to the mammalian expression vector pDEST40, using the enzyme LR Clonase II.

*Cell Culture/Transfection.* HEK 293T cells were maintained in DMEM (Gibco) with 10% FBS. The cells were cultured at 37°C and 5% CO<sub>2</sub>. One day prior to transfection of HEK293T cells with cDNA, the cells were plated in 6-6 well dishes (this was completed so the cells would be 50% confluent on day of transfection. Two plates were transfected with zCOX-2a N594A/pcDNA3.1 zeo+, two with zCOX-2a V434I/N594A/pcDNA3.1 zeo+, and two with zCOX-2a V523I/N594A. 2 µg of DNA and 5 µl of Lipofectamine 2000 were used per well. The cells were incubated overnight and a metabolism study was performed following day.

*COX Inhibitor Assay.* Transfected cells were tested with five different concentrations of inhibitor (plus a DMSO control). Inhibitors used for assay were rofecoxib (Vioxx), celecoxib (Celebrex), ibuprofen, indomethacin, flurbiprofen. Rofecoxib, celecoxib, LM-5201, and LM-4108 (COX-2 selective inhibitors; LM- lab synthesized) were used on both wild type and mutant zebra fish COX-2. Ibuprofen, indomethacin, and flurbiprofen (non-selective inhibitors) were only treated on wild type zebra fish COX-2. Arachidonic acid was used as the substrate.

*LC-MS/MS.* Samples were sent to Vanderbilt's Mass Spec. lab to be analyzed. Chromatographic separation of the PGE<sub>2</sub> and PGD<sub>2</sub> was achieved on an Acuity UPLC BEH C18 column (2.1 x 50 mm, held at 40°C) with isocratic elution for 3 minutes utilizing 60 % solution A (5 mM ammonium acetate, pH 3.5) and 40% solution B (acetonitrile with 10% solution). Mass spectrometric analysis was performed on a Thermo Quantum triple quadruple mass spectrometer. A Thermo Surveyor and autosampler and

pump, in-line with the mass spectrometer, were used for LC-MS analysis. PGE<sub>2</sub> and PGD<sub>2</sub> were detected via selected reaction monitoring (SRM) (as [M-H]<sup>-</sup> complexes) in the negative ion mode using the following reactions (the mass in parentheses represents the mass of the deuterated internal standard): 351 (355) → 271 (275). Quantitation was completed by calculating the ratio of PGE<sub>2</sub> plus PGD<sub>2</sub> to PGE<sub>2</sub>d<sub>4</sub> (internal standard) (Cayman Chemical). Results were plotted as percent remaining activity for each inhibitor concentration as compared to the DMSO control.

### **Data Analysis**

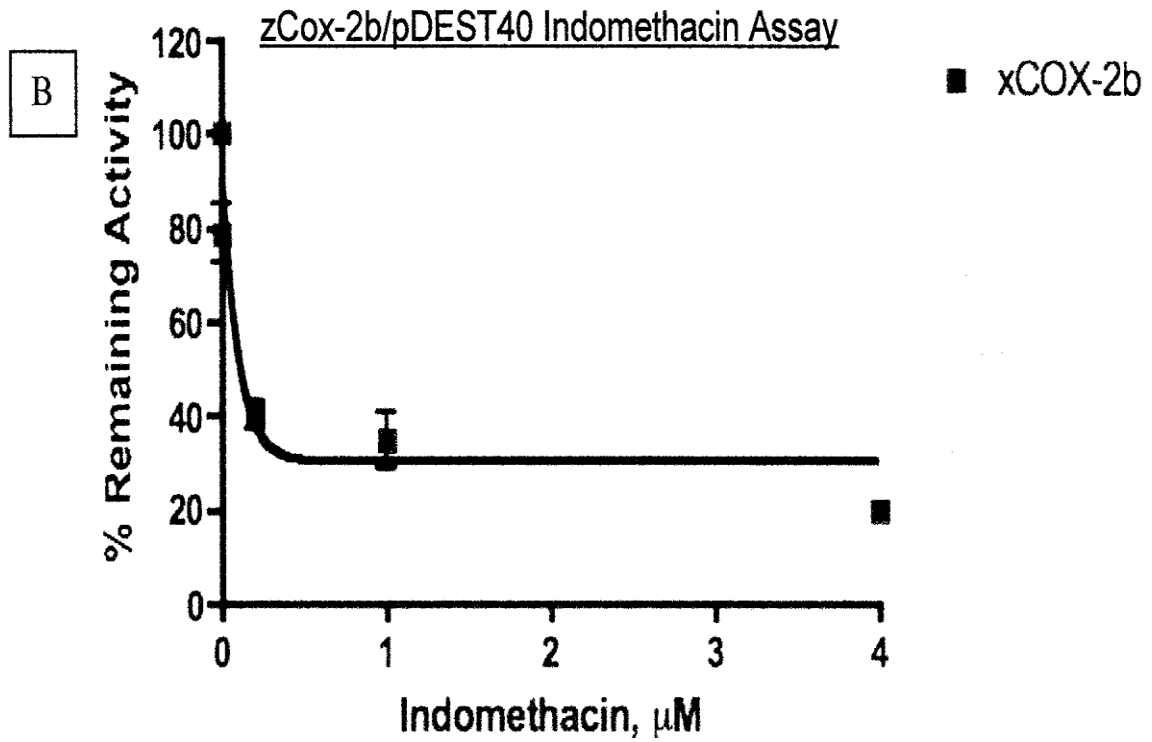
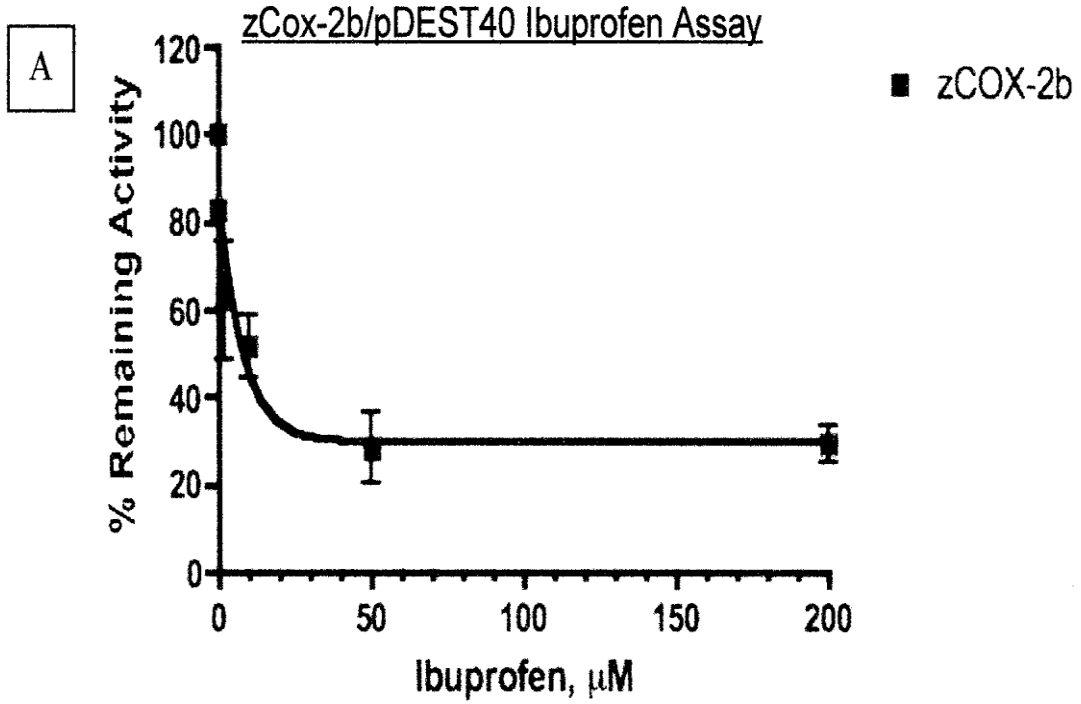
In figures A and B, HEK 293T cells transiently transfected with zCOX-2b/pDEST40 were treated with various concentrations of COX nonselective inhibitors ibuprofen (0-200 μM) (A) or indomethacin (0-4 μM) (B) followed by addition of arachidonic acid. Prostaglandin E<sub>2</sub> and D<sub>2</sub> levels were determined by LC-MS/MS analysis, and results were plotted as percent remaining activity as compared to DMSO control. Both indomethacin and ibuprofen efficiently reduced the activity of prostaglandins E<sub>2</sub> and D<sub>2</sub>, retaining IC<sub>50</sub> values of 87 nM and 11 μM respectively.

In figures C-F, HEK 293T cells transiently transfected with zCOX-1 or -2a/pcDNA3.1 zeo+ were treated with various concentrations of COX-2 selective inhibitors rofecoxib (0-100 μM) (C,D) or celecoxib (0-50 μM) (E,F) followed by addition of arachidonic acid. Figure C shows that rofecoxib is a poor inhibitor of zCOX-1 and zCOX-1 V523I (IC<sub>50</sub> >100 μM in both cases). In figure D, rofecoxib proved to be an excellent inhibitor of zCOX-2a (IC<sub>50</sub> 50nM). When the Valine located at residue 434 in zCOX-2a was mutated to an Isoleucine, the IC<sub>50</sub> slightly increased to 1.65 μM; however,

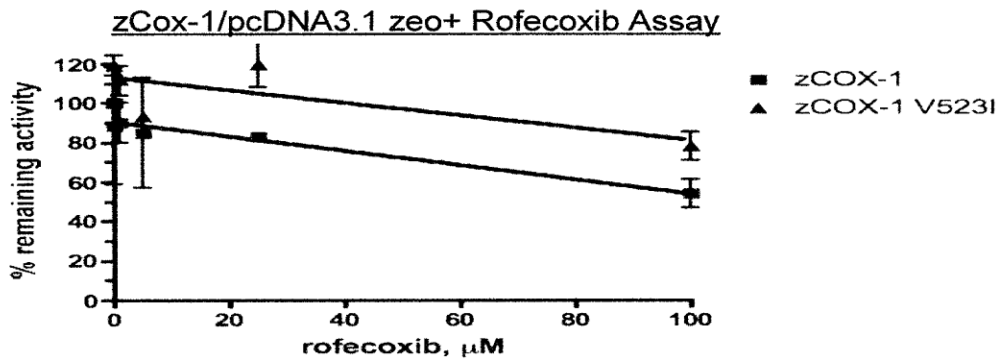
when the Valine at residue 523 was mutated to an Isoleucine, the  $IC_{50}$  value drastically increased to  $>30 \mu\text{M}$ .

Figures E and F plot the percent remaining activity in the standard and two mutations of the isoform zCOX-2a when treated with COX-2 selective inhibitor celecoxib. In figure E, zCOX-1 was inhibited by celecoxib with an  $IC_{50}$  value of  $4.8 \mu\text{M}$ ; when the Isoleucine at residue 434 was mutated to a Valine, this value decreased slightly ( $3.4 \mu\text{M}$ ). However, celecoxib was a much more efficient inhibitor of COX-2a, holding an  $IC_{50}$  value of  $450 \text{ nM}$ . When the Valine at residue 434 was mutated to Isoleucine, the  $IC_{50}$  increased slightly to  $1.65 \mu\text{M}$ ; however, there was a much larger response when the Valine at residue 523 was mutated to an Isoleucine, with the  $IC_{50}$  increasing to  $>30 \mu\text{M}$ .

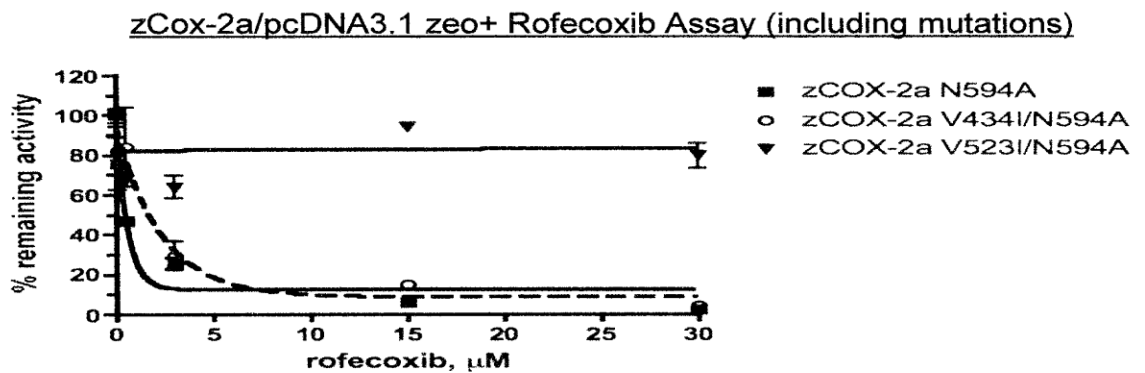
Graphical Mass Spec Analysis of Inhibitory Assays



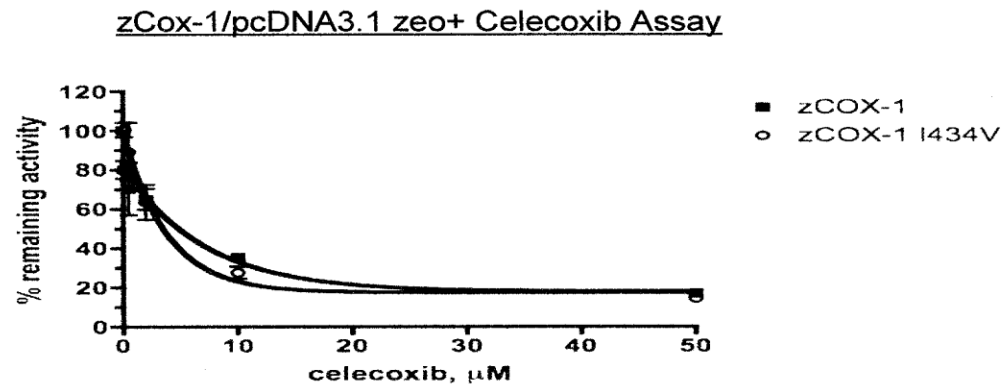
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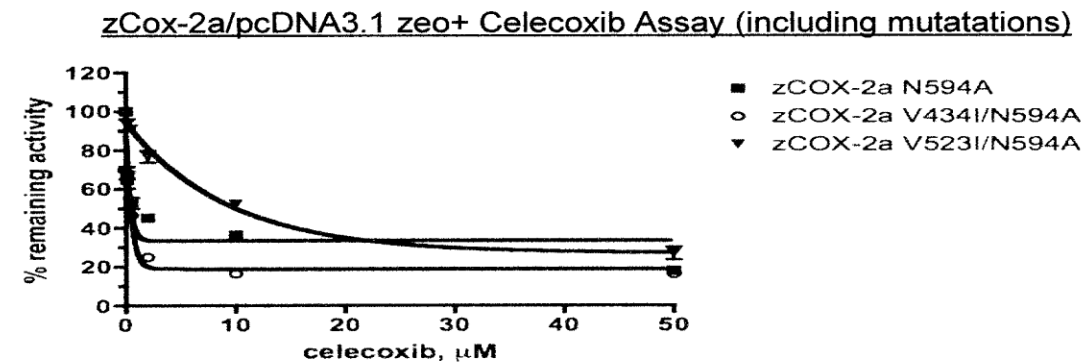
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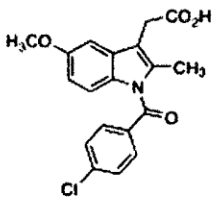
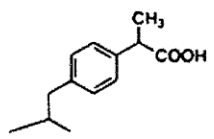
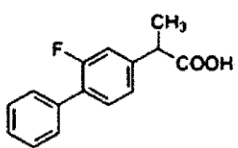
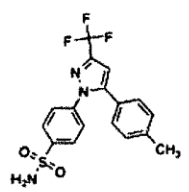
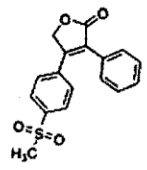
E



F



## IC<sub>50</sub> Values

Compound	Isoform	IC <sub>50</sub>
	zCOX-1	87 nM
	zCOX-2a	110 nM
	zCOX-2b	110 nM
	zCOX-1	11 μM
	zCOX-2a	6.2 μM
	zCOX-2b	8.1 μM
	zCOX-1	160 nM
	zCOX-2a	84 nM
	zCOX-1	4.8 μM
	zCOX-1 I434V	3.4 μM
	zCOX-2a	450 nM
	zCOX-2a V434I	390 nM
	zCOX-2a V523I	10 μM
	zCOX-1	>100 μM
	zCOX-1 V523I	>100 μM
	zCOX-2a	50 nM
	zCOX-2a V434I	1.65 μM
	zCOX-2a V523I	>30 μM

## Conclusion

In conclusion, the original hypothesis that the valine located at residues 434 and 523 in zCOX-2a, and the isoleucine located at residue 434 in zCOX-1 play an important role in the binding of inhibitor to zCOX-2a was partly correct. This original hypothesis was established on the basis of molecular research of the cyclooxygenase active site. These two amino acids are the most influential on the difference in size and shape of the zCOX-2 enzyme as compared to the zCOX-1, providing an approximately 20% larger active site and more substrate flexibility for zCOX-2 (Smith, 2000).

Referring to the graphs (A-F) and the IC<sub>50</sub> value chart, graphs A and B display the inhibition of zCOX-2b enzymes by nonselective inhibitors (ibuprofen and indomethacin). The main purpose of this trial was to show that zCOX-2b is indeed inhibited by nonselective inhibitors. Little else is known about the mechanisms behind zCOX-2b, therefore this trial may be used as a future direction note for further research regarding cyclooxygenase enzymes.

In graphs C and D, the COX-2 specific inhibitor rofecoxib (Vioxx) was used to see how it inhibited both zCOX-1 and zCOX-2a. Graph C shows that zCOX-1 enzyme is inhibited to a small extent by rofecoxib. In fact, the IC<sub>50</sub> value for zCOX-1 with rofecoxib is >100μM. This value is so large that COX-1 inhibition by rofecoxib is not even recognized (COX-1 levels maintained). Similar results occurred when the valine at residue 523 was mutated to isoleucine, showing that this valine plays no important role in the binding of COX-2 specific inhibitor to zCOX-1. Graph D explores the inhibition of zCOX-2a by rofecoxib. For the control, zCOX-2a was inhibited by rofecoxib with an IC<sub>50</sub> value of 50nM (this value indicates excellent inhibition). When the valine at residue 434



was mutated to isoleucine, the  $IC_{50}$  value slightly increased ( $1.65\mu\text{M}$ ) indicating that this amino acid plays a relatively unimportant role in the binding of COX-2 specific inhibitor to zCOX-2a; however, when the valine at residue 523 was mutated to isoleucine, a much larger response was noted. The  $IC_{50}$  value increased drastically ( $>30\mu\text{M}$ ), indicating that this amino acid plays a much more important role in the binding of inhibitor to COX-2a.

Graphs E and F basically serve the same purpose as C and D, except celecoxib (Celebrex) was used as the specific inhibitor instead of rofecoxib. Graph C also displays that when the isoleucine at residue 434 is mutated to valine, little changes in the  $IC_{50}$  value, showing that the isoleucine plays a relatively unimportant role in the binding of inhibitor to zCOX-1. Additional information gathered from this graphic corresponds to conclusions drawn from experimentation with zCOX-2 and celecoxib (valine at 434 plays an unimportant role; valine at 523 plays an important role).

From the experimentation with cyclooxygenase enzymes and inhibitors many conclusions can be drawn. The valine located at 434 for zCOX-2a plays an unimportant role in the binding of inhibitor to zCOX-2a. On the other hand, the valine located at residue 523 does play an important role in the binding of inhibitor to zCOX-2a. Finally, contrary to mammalian COX isoforms, Ile-434 and Val-523 appear to have no significant role in the binding of COX-2 selective inhibitors to zCOX-1. These discoveries broaden the horizon for future direction in cyclooxygenase research. Specifically, future experimentation may be completed to consider the roles of amino acid sequencing in the binding of COX-2 specific inhibitor to zCOX-1 and zCOX-2b (Cha, 2006). This future research may, most importantly, be used as an exploit for future drug design in the category of non-COX specific NSAIDs.

## **Acknowledgement**

Special thanks to Dr. Robyn Jannetta and Dr. Lawrence J. Marnett of Vanderbilt University (Marnett Research Laboratory, Vanderbilt University) for research assistance and use of laboratory materials.

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# **The Dissolution Rate of Non-Narcotic Analgesics to Determine Efficacy Under Simulated Stomach Conditions**

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## **Abstract**

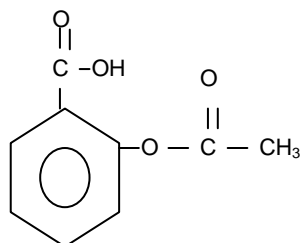
Non-narcotic analgesics used for short-term pain relief fall into four categories: aspirin, ibuprofen, acetaminophen, and naproxen. Since ingestion is the method of delivery to the body, breakup of these materials in the stomach is essential for distribution via the blood stream. In this research commercially available samples of all these materials were placed in pH 2.0 solution at 37°C, and the breakup time and effect on pH were determined. The powdered analgesics have the shortest breakup time and the gelled capsules and caplets had the longest breakup time. Buffered analgesics neutralized the solution acidity and significantly raised pH while the acidic, non-buffered analgesics significantly decreased the solution pH. This research further showed that powdered forms of the analgesics would provide the fastest pain relief.

## **Introduction**

Analgesics are divided into two categories: narcotic and non-narcotic. Narcotics are mostly used on a short-term basis for severe pain. They act on the central-nervous system (CNS) and if used over an extended period of time, may become habit forming (3). Some examples of drugs that fall into the narcotic category are opium, codeine, and morphine. Non-narcotics, on the other hand, are most often used to alleviate common pains. They do not act upon the CNS as the narcotics do, and therefore, are safer to use.

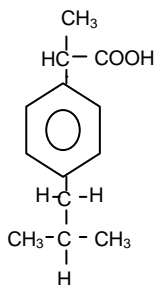
Examples of drugs that fall into the non-narcotic category are aspirin, ibuprofen, acetaminophen, and naproxen.

Aspirin (acetylsalicylic acid), known by names such as Bayer and Ecotrin, are a derivative of salicylic acid. On August 10, 1897, Felix Hoffmann, founder of Bayer, converted salicylic acid with an acetyl group into acetylsalicylic acid, which is gentler on the stomach (5). The body absorbs this material and converts it back to salicylic acid, which is ultimately filtered by the kidneys and liver into the urine. While in the body, it reacts with cyclooxygenase (COX) enzymes 1 and 3, which are found in normal tissue, and stops them from sending pain signals to the brain. There are reported side effects, varying from minor heartburn and upset stomach to severe gastrointestinal bleeding (4).



Aspirin (acetylsalicylic acid)

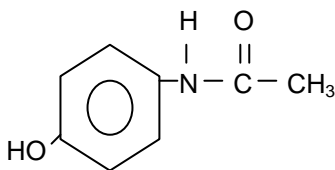
Ibuprofen is a non-steroidal anti-inflammatory drug (NSAID), known by brands names Advil and Motrin (7). It works by reducing hormones that cause inflammation and pain in the body. Like aspirin, if an overdose occurs, it may cause damage to the stomach and intestines (7). If a person has cardiac disease, side effects would prohibit the use of this drug.



Ibuprofen

((±)2-(4-isobutylphenyl) propionic acid)

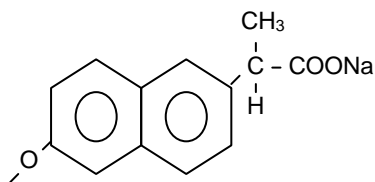
Acetaminophen, known by brand names such as Tylenol and Midol, lacks the anti-inflammatory and blood-thinning effects that aspirin and ibuprofen produce (6). Because it does not cause stomach and intestinal ulcers, it is used by people who cannot tolerate NSAIDs (6). However, the patient should exercise caution because an overdose may lead to liver failure. It works on the COX-3 enzyme which is present in the brain and spinal cord to stop all prostaglandin activities in the body.



Acetaminophen

(4'-hydroxyacetanilide)

Naproxen is also an NSAID known by the brand names Aleve and Anaprox (2). Similar to ibuprofen, it reduces prostaglandins in the body, and possesses the same side effects. It is used primarily to treat arthritis, tendonitis, and pain associated with menstrual cycles (1).



Naproxen Sodium  
(2-naphthaleneacetic acid,  
6-methoxy- $\alpha$ -methyl-sodium salt)

All of the substances presented here (aspirin, ibuprofen, acetaminophen, and naproxen) are of the non-narcotic category of analgesics and are mostly used for common pain reduction.

Presumably, any drug such as these analgesics must be either aqueous or lipid soluble in the stomach in order to enter the system via absorption to become effective. This research project was done in order to determine the rate at which non-narcotic analgesics dissolve under simulated stomach conditions in the laboratory. Each of these drugs is compounded with fillers such as  $\text{TiO}_2$  and/or buffers such as  $\text{Ca}(\text{OH})_2$ , along with capsule or gel cap components. These materials often do not dissolve even at pH 2.0 (typical stomach pH) so “break-up” rate was measured as opposed to actual dissolution.

### **Methods and Materials**

Each of the four types of analgesics was used in this research. Bayer (chewable and tablet), Bufferin, and B.C. Powder represented aspirin. Tylenol, Midol, and Goody’s Powder represented acetaminophen. Motrin, and Advil (liqui-gel and tablet) were the ibuprofen brands. Naproxen Sodium, and Aleve, liquid-gel, were selected as examples of naproxen.

The pH of 100 mL 0.01 M hydrochloric acid (HCl), calculated to be 2.0, was measured with a Hanna Instruments pH 211 Meter equipped with a combination electrode, 2-buffer standardized with pH 4.0 and 7.0 commercial buffers. This solution was then heated to approximately 37°C to simulate normal body temperature.

Once the solution was at the desired temperature and the initial pH determined, one tablet, capsule, or powder of the chosen sample was added to the HCl solution with stirring by a magnetic stirrer. The time until complete breakup of the sample was determined. For most of the samples some insolubles (fillers and other additives), rendered the mixture cloudy.

After the sample had fully dispersed, and the time recorded, the pH level was again measured and the pH difference was calculated.

### **Results and Discussion**

All of the samples of analgesics contain substances other than the active ingredient. Table 1 lists the types, brands, and active dosages, along with the mass of the additives. These additives take the form of insoluble fillers, pH buffers, and encapsulating materials. The dosage generally is about one-half of the total mass of the substances except for only a few: Bayer tablets, Midol, Advil Liqui-Gel, and Aleve Liquid. When dispersed in the HCl solution, the fillers caused the mixture to be cloudy indicating that dissolution of those materials did not occur.

**Table 1****Non-Narcotic Analgesics Dosage and Mass (g)**

Type	Brand	Dosage (mg)	Total Mass (mg)	Additives (mg)
Aspirin	Bufferin Bayer	325	668	343
Aspirin	Chewable	81	230	149
Aspirin	Bayer Tablets	325	394	69
Aspirin	B.C. Powder	650	1064	414
Acetaminophen	Tylenol Tablets	325	395	70
Acetaminophen	Midol	500	650	150
Acetaminophen/ Aspirin	Goody's	780	1164	384
Ibuprofen	Motrin I.B.	200	317	117
Ibuprofen	Advil Liqui-Gels	200	835	635
Ibuprofen	Advil	200	472	272
Naproxen Sodium	Aleve Liquid- Gels	220	1362	1142
Naproxen Sodium	Generic	220	308	88

Most of the analgesics used had some effect on the pH levels. Bufferin, Aleve, and the Generic Naproxen Sodium partially neutralized the acid. Table 2 shows the mean and standard deviation ( $\sigma$ ) for the pH difference before and after adding the sample to the HCl solution. Figure 1 also shows that the analgesics used raised or lowered the pH depending on whether or not a weak base was a component of the formulation. Figure 1 shows that Advil Liqui-Gel, Naproxen Sodium, and Bufferin increased the pH which becomes a negative change in the chart indicating that these materials decrease stomach acid. The others tested increased stomach acidity to varying extents.

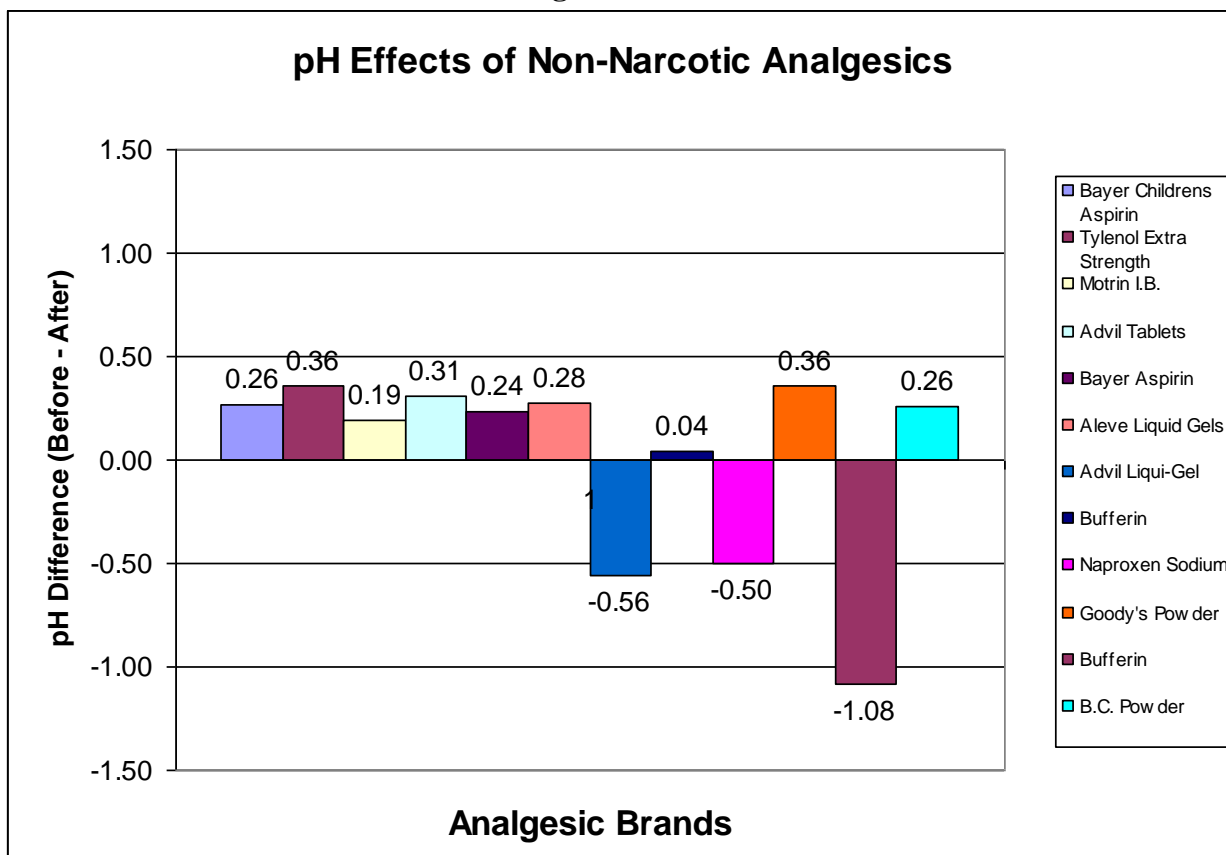


**Table 2**

**Non-Narcotic Analgesics Effects on pH and Breakup Time**

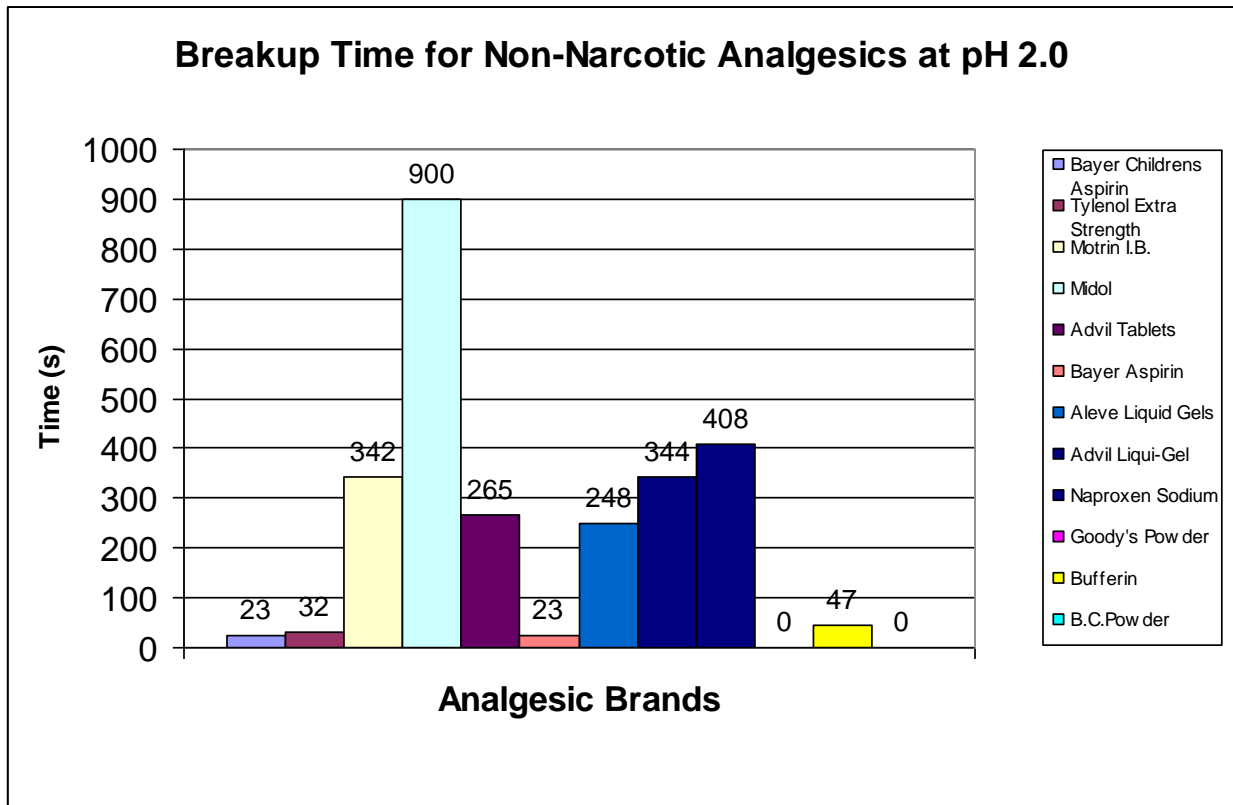
Type	Brand	pH Difference		Breakup Time (s)	
		Mean	$\sigma$	Mean	$\sigma$
Aspirin	Bufferin	-1.07	0.15	47	8
Aspirin	Bayer Chewable	0.26	0.11	23	2
Aspirin	Bayer Tablets	0.28	0.07	23	1
Aspirin	B.C. Powder	0.23	0.04	0	0
Acetaminophen	Tylenol Tablets	0.36	0.14	32	3
Acetaminophen	Midol	0.31	0.30	900	51
Acetaminophen	Goody's	0.36	0.13	0	0
Ibuprofen	Motrin I.B.	0.19	0.10	342	26
Ibuprofen	Advil Liqui-Gels	0.04	0.11	344	58
Ibuprofen	Advil	0.24	0.04	265	26
Naproxen Sodium	Aleve Liquid-Gels	-0.56	0.03	248	183
Naproxen Sodium	Generic	-0.50	0.31	408	21

**Figure 1**



Since the samples tested were composed of different materials and produced by different manufacturing methods, there was also a considerable difference in break up times. Table 2 and Figure 2 show that B.C. Powder and Goody's took no time to break up, for both were already in powder form. Bayer Chewable, Bayer Tablets, and Tylenol were loosely formed into pills, and broke up in roughly thirty seconds. The products that took the form of caplets or gel capsules required 248 to 900 seconds for complete break up in the HCl.

Figure 2



## **Conclusions**

The over-the-counter, non-narcotic analgesics certainly have been proved effective in common pain relief as evidenced by their widespread use throughout the world. This fact has resulted in a proliferation of brands and types available. The research conducted here was done to determine how fast one could expect pain relief to occur after ingestion. The guiding premise was that dissolution time would be proportional to the speed of stomach absorption, and therefore, also proportional to the speed of relief. However, since complete dissolution did not occur in any of the samples due to insoluble components, the break-up time became the measurable variable.

The fastest acting pain relievers would be those that begin as powdered products (Goody's Powder, and B.C. Powder). The slowest acting would be those beginning as caplets or gel capsules such as Midol and Naproxen Sodium. However, the popularity of the materials appears more related to taste than to speed of action to make the use of caplets and gel capsules more widespread.

Buffering action of the products is also an important consideration. Slight buffering of the stomach acid would be beneficial in mild cases of indigestion. In this case Advil Liqui-Gel, Goody's Powder, and B.C. Powder would all be effective.

## **Future Direction**

Expanding this research should include actual absorption rate, and could be accomplished by using semi-permeable membranes that would simulate the stomach lining in its absorptive ability.

## **Acknowledgements**

I would like to thank my parents for helping me accomplish all that I have done for this project. I would also like to thank Mr. Ronnie Nixon, my science teacher, for assisting me with all the information and experiments for this project.

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# **Implementation of a Tricyclic Antidepressant on *C. elegans* Mutants and Its Effects on Locomotion**

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## **Abstract**

Imipramine primarily targets dopamine and serotonin transporters in *C. elegans*, blocking reuptake. We tested mutants, each lacking a specific neurotransmitter, to measure locomotion after exposure to Imipramine. We hypothesized that if neurotransmitter interactions were minimal and an octopamine transporter was not present, then mutants containing target transporters would paralyze, while others would remain motile. Our hypothesis held true, except for the tyramine-octopamine (*tdc-1*) mutants, which were unaffected despite presence of dopamine and serotonin. These results were confirmed in a swimming assay. We believe there are two possible explanations of the results: *either* tyramine and octopamine augment the production of target neurotransmitters *or* a novel octopamine transporter is present.

## **Introduction**

*Caenorhabditis elegans*, a nematode, is a commonly used model organism. This model has been used to study a range of scientific endeavors ranging from neuroscience to genetics. Researchers working with *C. elegans* as a mode of studying neuroscience have identified and easily manipulated the production pathways of many neurotransmitters, which warrants its frequent usage in science. Neurotransmitters are transferred by transporter molecules; the most common being dopamine, serotonin, tyramine, and

octopamine. Researchers working with *C. elegans* have often speculated of an octopamine transporter (which is present in other organisms such as humans), but have been unsuccessful in finding one (Roeder, 2005). *C. elegans* researchers also use this organism as a mode of preliminary drug testing: specifically antidepressants.

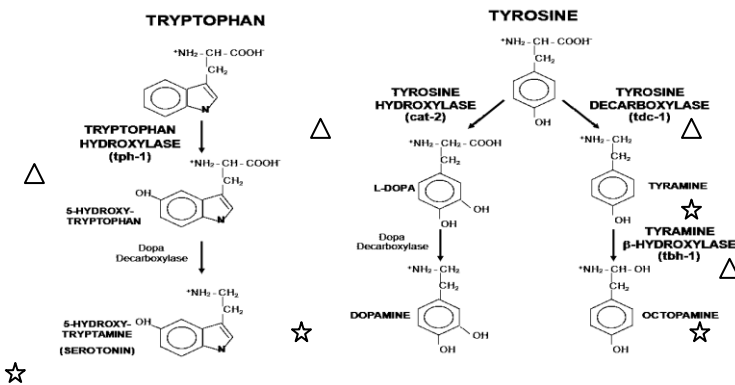
One of the initial antidepressants created belongs to a class of tricyclic antidepressants, and was used to treat disorders such as schizophrenia, bipolar complexes, and depression (Krishnan, 2008). This drug, Imipramine, is currently out of practice and has been replaced by modern antidepressants such as Prozac; however, it is still referred to as the “Gold Standard” antidepressant because of its potency (Hardie, pers. comm.). Imipramine as a monoamine inhibitor primarily targets the dopamine and serotonin transporters, blocking the post synaptic receptor (Chase, 2007; Petrascheck, 2007). This causes an accumulation of the two neurotransmitters (dopamine and serotonin) in cells, leading to paralysis in *C. elegans* (Chase, 2007). *C. elegans* containing these target neurotransmitters, or their respective neurotransporters should be affected by the drug, because reuptake will not occur despite the loss of other neurotransmitters that Imipramine has no effect on. However, if non target neurotransmitters do interact with target molecules, mutants which still contain target transporters should become paralyzed due to a change in dopamine and serotonin concentration or the presence of an octopamine transporter. Therefore, we hypothesized that the properties of Imipramine would hold true, and neurotransmitter interaction would not affect the paralysis of the organism, and by extension an octopamine transporter would not be present.

## Methods

### Derivation of Mutants

For each mutant line of *C. elegans*, the gene that is essential for the primary pathway production for the various amines was genetically inactivated, and then obtained as a generous gift from the Blakely Lab from Vanderbilt. For the *cat-2* mutant, the *tyrosine hydroxylase* (Figure 1) gene was inactivated which severely reduced the production of dopamine. Previous studies have shown that by knocking out the *tyrosine hydroxylase* gene has reduced dopamine production up to 70% rather than full eradication because alternative pathways of dopamine production exist (Sanyal, 2004). Similarly, the *tph-1* mutant was derived by inactivating the *tryptophan hydroxylase* gene (Figure 1) which consequently reduced the presence of serotonin in *C. elegans*. The *tbh-1* mutant was produced by inactivating just the *tyramine β-hydroxylase* gene (Figure 1) which reduced octopamine production: and the *tdc-1* mutant was derived by silencing the *tyrosine decarboxylase* gene (Figure 1) which subsequently reduces the production of both tyramine and octopamine.

The Biogenic Amines



Population	Mutation
<i>N2</i>	Wildtype-none (Positive Control)
<i>dat-1</i>	Lacking Dopamine Transporter (Negative Control)
<i>cat-2</i>	Dopamine Deficient
<i>tbh-1</i>	Octopamine Deficient
<i>tdc-1</i>	Octopamine/Tyramine Deficient
<i>tph-1</i>	Serotonin Deficient

**Table 1:** A summary of the various mutant names and their deficiencies

**Figure 1:** A depiction of the biogenic amines pathways

Δ : Gene that controls amine production

☆ : Biological amine produced

\*Figure from the Blakely Lab at Vanderbilt University, Nashville, TN

### **Imipramine on NGM Plates**

To gather our results, we performed two different assays using Imipramine. For our first assay, we spread 100 $\mu$ l of a 1.25mM Imipramine solution uniformly on Normal Growth Medium (NGM) plates. The solution was allowed to dry on the plates to become an overlay on the NGM plates. After the solution dried on the plates, we placed 10 individual worms in the L3 to adult stage from each population, on designated plates. We incrementally checked the state of individual's motility in 1 minute intervals until the whole population was paralyzed. If, however, the population was not paralyzed, we capped our time at 10 minutes, assuming that the motile individuals were not paralyzed by the drug. One individual from each population was observed under a recording microscope to obtain qualitative data showing the paralysis of various mutants.

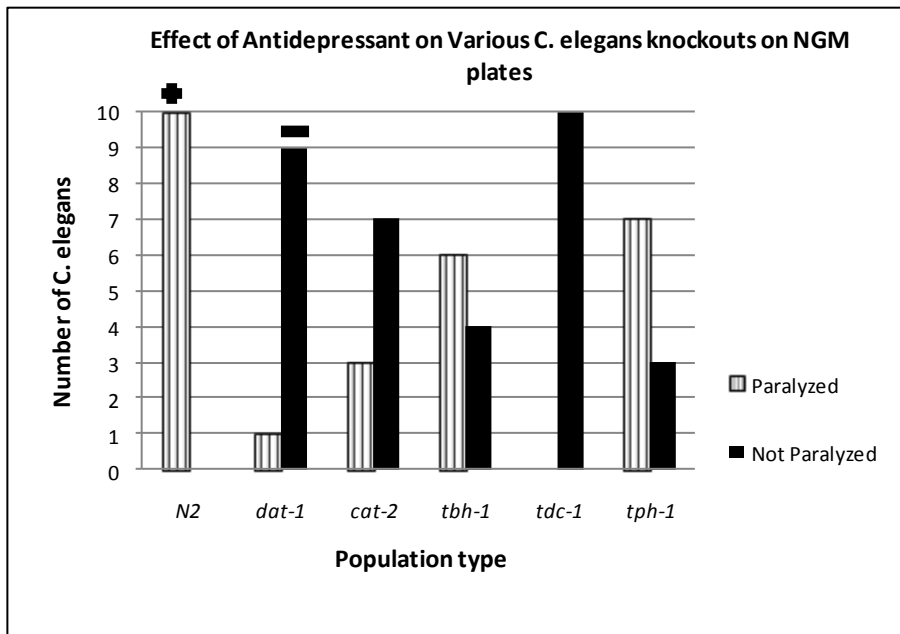
### **Swimming Assay**

To confirm our results in the previous assay, we performed a swimming assay with the same mutants. In this experiment, we used 3 x 3 well plates and placed the *C. elegans* in the L3 to adult stage (5 from each population) in 80 $\mu$ l in a 1mM (diluted with 50 mOsm H<sub>2</sub>O) solution of Imipramine, as done by previous studies (Hardie, pers. comm.). When *C. elegans* is placed in these wells, their natural reflex is to swim by thrashing violently (Hardie, pers. comm.). Because Imipramine inhibits locomotion, the time it takes individuals to become paralyzed is representative of Imipramine's extent of effectiveness. Once the individuals were placed in the wells, we observed their locomotion in 1 minute increments and recorded paralysis. As in the previous assay, we capped our time at 10 minutes.



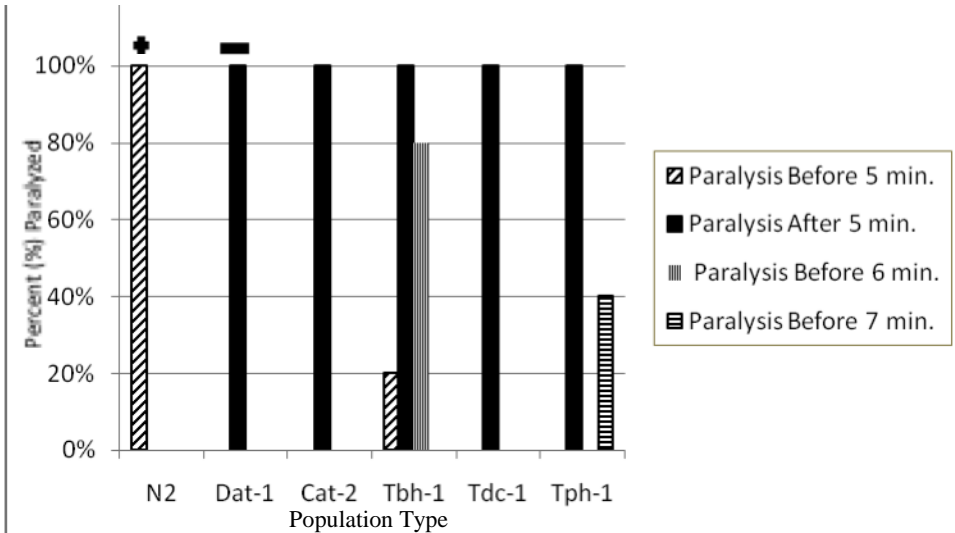
## Results

First, we determined the extent to which Imipramine affected the various mutants on NGM plates. Figure 2 shows that the majority of the *N2*, *tbh-1*, and *tph-1* populations paralyzed in the presence of Imipramine, while the majority of the *dat-1*, *cat-2*, and *tdc-1* populations remained motile.



**Figure 2:** Data showing rate of paralysis in various populations on NGM plates.  
+ : positive control  
■ : negative control

In order to confirm the results from our first test, we performed a swimming assay to time the paralysis of the various populations. In this test, we saw that the wildtype population paralyzed the fastest, followed by the *tph-1*, then *tbh-1* (Figure 3). The *dat-1*, *cat-2*, and *tdc-1* populations did not paralyze until after 7 minutes (Figure 3).



**Figure 3 Effects of Imipramine on Various *C. elegans* mutants in solution:**

Results showing paralysis in Imipramine solution

◆: positive control    ■: negative control    n=5 worms per population

### Discussion

The overall purpose of this experiment was to find the extent of neurotransmitter interactions, or the possible presence of an octopamine transporter. As stated previously, Imipramine blocks dopamine and serotonin post-synaptic junctions, causing a buildup of these neurotransmitters (Chase, 2007; Petrascheck, 2007). Consequently, this buildup results in paralysis of the organism (Chase, 2007). Based on this, we hypothesized that mutants containing the target neurotransmitters would become paralyzed, and those lacking dopamine and serotonin would remain motile. Based on the data in Figures 2, our hypothesis held true for all mutants except the *tdc-1* population. The positive control acted as expected because the wildtype population, containing target neurotransmitters, became paralyzed. The negative control, *dat-1*, which lacks the dopamine transporter, remained motile because it lacks the transporter that Imipramine blocks, rendering the drug

ineffective. These positive and negative controls assured our group that the Imipramine solution was at an adequate potency, confirmed that Imipramine acts as expected on *C. elegans*, and verified the derivation process. The results for *tph-1* and *cat-2* show us that Imipramine targets dopamine more heavily than serotonin. This can be inferred because in the presence of Imipramine, 70% of the *cat-2* mutants (lacking 70% of their dopamine) remained motile (Sanyal, 2004). Conversely, when serotonin was removed from *C. elegans*, 70% of the organisms became paralyzed, as dopamine was still present in these mutants' systems. For *tbh-1*, only 60% of the organisms paralyzed despite the presence of both dopamine and serotonin. It is noteworthy to say that for this mutant, there have been studies that have shown that octopamine deficient mutants contain 10 times the amount of tyramine in their systems because the pathway to convert tyramine into octopamine does not exist (Figure 1) (Hardie, pers. comm.).

Finally, 100% of the *tdc-1* mutants (lacking both tyramine and octopamine) unexpectedly remained motile, and were unaffected by Imipramine. This result was very surprising because target neurotransmitters were still present in this system; and despite this, the population still was not paralyzed. From this, we infer that there are neurochemical interactions between tyramine, octopamine, dopamine, and serotonin.

After our initial assay, we performed a swimming assay to confirm our results. The results in Figure 3 mimic our results from Figure 2 in a more emphatic manner because there is greater exposure of Imipramine to *C. elegans*. Therefore, 100% of the *dat-1* and *cat-2* took longer than 10 minutes to paralyze, and all of the *N2* mutants paralyzed within 5 minutes. The *tph-1* mutants took longer to paralyze (between 5 and 7 minutes) because of its presence of dopamine, but deficiency in serotonin. The rate of paralysis for *tbh-1*

was very sporadic, and included mutants that paralyzed before 5 minutes, between 5 and 6 minutes, and still others that did not paralyze. Finally, the entire *tdc-1* population remained motile, even after 15 minutes of observation. The sporadic nature of the *tbh-1* results shows that the increased concentration of tyramine affects the properties of dopamine and serotonin, and therefore, the population's motility. The *tdc-1* results support our previous assessment that dopamine, serotonin, tyramine, and octopamine interact.

Our results show that there are interactions between the four neurotransmitters, dopamine, serotonin, tyramine, and octopamine. From here, one could create double mutants to test the validity of this hypothesis, test various antidepressants to illicit different neurological effects, and use proteomic analysis to search for a putative octopamine transporter. In the future, these results could be used to perform more refined antidepressant and *C. elegans* research.

### **Acknowledgements**

We would like to thank Dr. Hardie and the Blakely lab for supplying material, time, and tutoring. We are also grateful for Drs. Eeds, Creamer, McCue, and Vanags for their support and advice throughout this research project. Finally, we would like to thank Aziza Hart and Kevin Roman for their collaboration, lab work, and effort throughout these experiments.

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# **Determining the Efficiency of the EPA's Rapid Bioassessment Protocol for Nashville, Tennessee**

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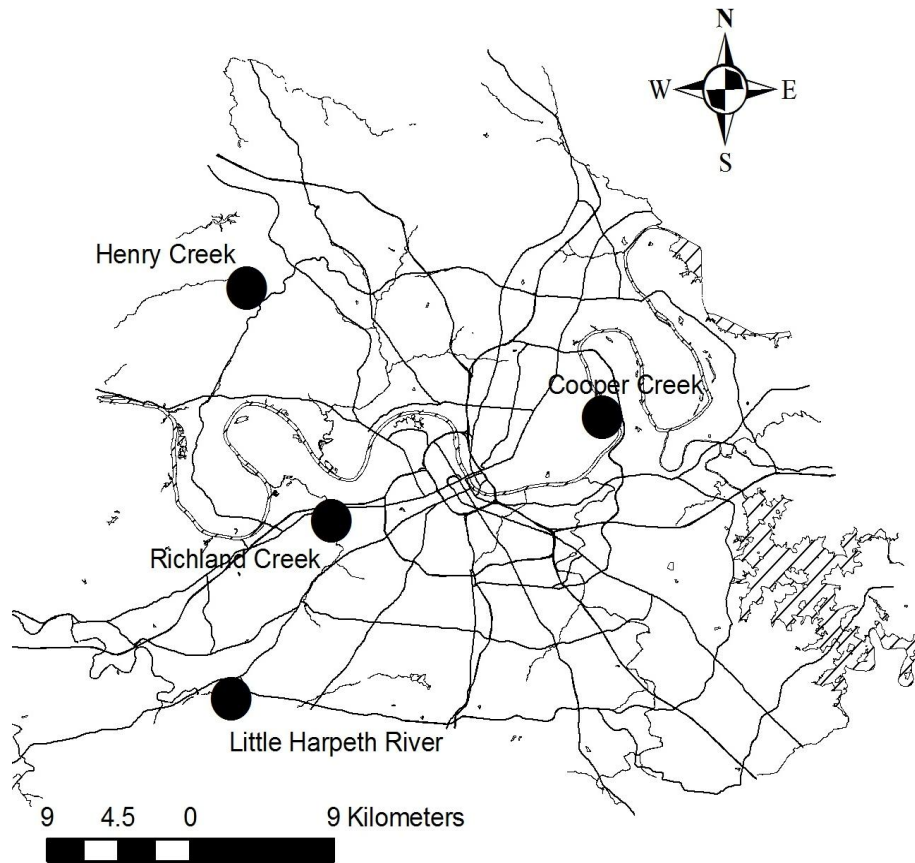
## **Abstract**

A previous survey of the water quality of Nashville streams determined that outside sources were degrading the quality of the streams. We performed assessments of four streams in order to compare the qualitative methods used by the EPA's Rapid Bioassessment Protocol to our quantitative methods of determining stream quality. Our quantitative methods measured pH, temperature, electrical conductivity and turbidity, and measured nitrate and phosphate levels at the beginning, middle, and end of the testing site. We collected macroinvertebrates to determine stream quality based on organism pollution tolerance. The RBP's methods were more efficient, but our methods were more accurate.

## **Introduction**

From previous studies performed in the Nashville area, it has been discovered that outside sources such as runoff, pollutants, and other environmental factors have a big effect on stream quality. We wanted to test stream quality because in previous studies of urban lakes around Nashville pollution was reported, and we thought tributaries could be the cause of this pollution, so we decided to test streams to continue our research. Next, we came across the EPA's Rapid Bioassessment Protocol, so we thought that comparing it to data that we could collect could assess the accuracy of this stream quality protocol. The EPA's Rapid Bioassessment Protocol (RBP) is a set of methods that are to be used to survey stream quality in the United States, but it is quite subjective in many aspects of its

scoring as stated by Dr. Barbour (Barbour 1992). The primary purpose of the RBP is to “describe a practical technical reference for conducting cost-effective biological assessments of lotic systems.” It doesn’t take into account a lot of the factors that make streams from separate regions different, such as area specific flora and fauna. Based on the subjectivity of the RBP, we decided to take quantitative water chemistry measurements from four streams in Nashville and compare our data to the composite score derived by completing the RBP. We performed both sets of methods on Henry Creek, Richland Creek, Cooper Creek, and Little Harpeth River. Each aspect of stream quality has its own individual effect on the health of the stream (Sanderson 2005). Temperature tends to create a healthier environment for plant and animal life when it is lower because high temperature decreases dissolved oxygen in water. Electrical conductivity (EC) is the ability of a material, in the case water, to carry an electrical current. EC is also a measure of the purity of water, since ions are what increase water’s ability to conduct electricity. Low nitrate levels are good because nitrates cause the inhibition of blood flow (Murphy 2007), and low levels of phosphates are good because phosphates can cause algal blooms which lower dissolved oxygen and can impact species diversity. Higher dissolved oxygen (DO) indicates better stream quality because all aerobic aquatic life needs high enough oxygen to survive. Acidic pH can lead to fewer organisms in a stream (Courtney 2008). Most of these aspects of stream quality are based on what the RBP measures in its analysis, but it is really hard to predict these measurements without direct data collection.



### Methods

All of our measurements took place from June 23<sup>rd</sup>, 2009 to June 26<sup>th</sup>, 2009. For each site, we began by recording the weather and environmental conditions, measuring out a 100m testing site, and drawing the survey area. We split into three groups to perform our set of quantitative methods first. Three of our members collected and identified macroinvertebrates by using a kick-net to gather them and using Hauer and Resh's identification key with a hand lens to identify them, tallied them up to roughly one hundred organisms, and assessed their pollution tolerance at each site. We obtained a weighted average macroinvertebrate pollution tolerance score for each of the four creeks



by taking the percentage of each species we collected out of 100, multiplying the percentage by its respective pollution tolerance, and summing them up to get a number between 1 and 10 (below)

$$\sum_{i=1}^s \frac{x_i}{n_i} * tol_i$$

Where  $s$  = number of species,  $n$  = number of organisms,  $x$  = number of organisms in the species, and  $tol$  = the organism's pollution tolerance score.

Higher tolerance values denote a high tolerance to pollution, and this can be an indicator of low stream quality, but not always. Two members used a multi-probe to measure the electrical conductivity, pH, and temperature at 5-meter intervals along the 100m testing site. The other two members performed nitrate and phosphate tests along the same 5-meter intervals. We took averages of pH, electrical conductivity, temperature, nitrates, and phosphates across the four creeks and compared each average to the creeks respective RBP score to assess how the RBPs component score relates to stream quality in each case. After completing our quantitative methods, we began performing the qualitative methods of the RBP on each stream. We performed a pebble count and a stream velocity test, and we measured stream gradient, bearing, and stream width. We also noted certain stream characteristics such as riparian width and canopy cover which can affect fauna of the streams as well as the amount of light, as well as heat, the streams get (Potter 2005). The composite RBP score was calculated by using the percentages of each aspect of the stream's environment, multiplying them by pre-established values provided by the protocol, and summing them up to get a total score out of 200. The RBP also had a section for quantifying macroinvertebrates, but so many pollution tolerance scores were missing

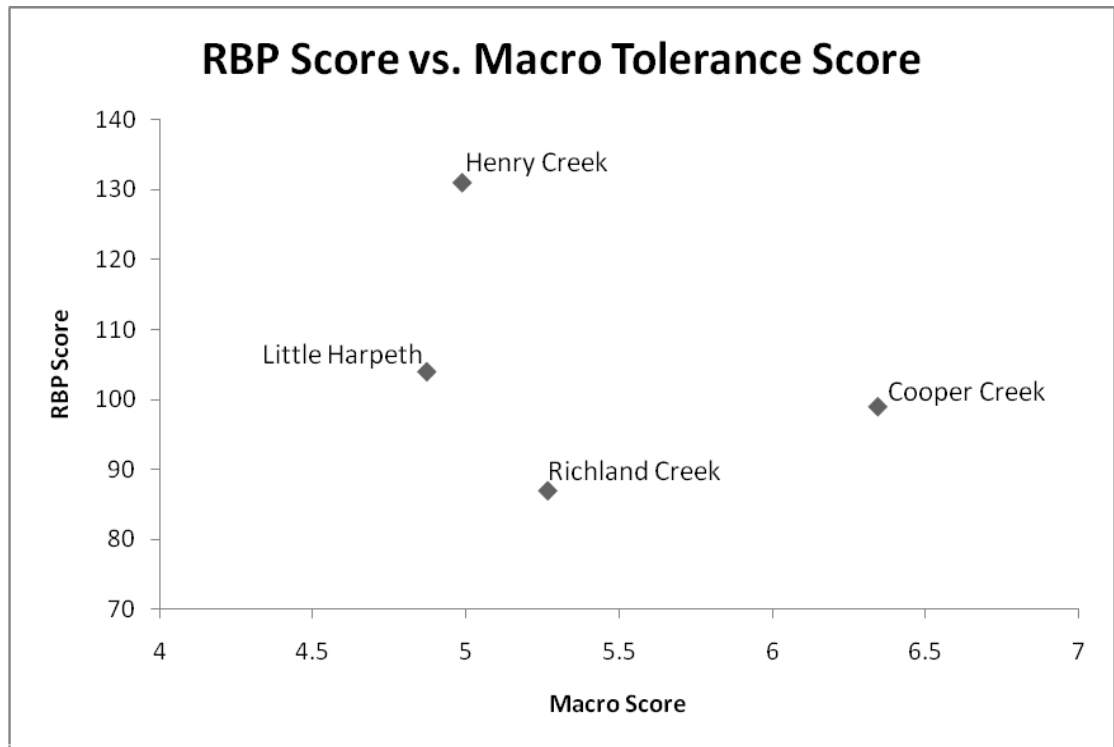
that it rendered that section unusable to our specific cause, therefore the organisms didn't tell us much about the stream quality.

### Results

Each aspect of quantitative data that we collected using our methods was compared to the component score of the Rapid Bioassessment Protocol for the four creeks (Table 1). In many cases, the nitrate levels in the water were below the detection limit of the equipment. Also, pH levels as well as phosphate levels tended to be similar across the four creeks. The graph of the macroinvertebrate tolerance versus the RBP score (Graph 1) seems to be the most important comparison since the living organisms of an environment are often the best indicator of environment quality. If the average pollution tolerance score for the macroinvertebrates, on a scale between 1 and 10, is low, that indicates good stream quality because it means that pollution intolerant organisms can thrive in the certain environment. The  $R^2$  value of Graph 1 is 0.27, which mean that there is very little correlation between RBP acquired stream quality and average tolerance of macroinvertebrates.

<u>Table 1</u>	<b>Cooper Creek</b>	<b>Little Harpeth</b>	<b>Henry Creek</b>	<b>Richland Creek</b>
Temperature (°C)	19.671	24.128	22.481	24.305
Dissolved Oxygen (mg/L)	12.333	8	7	9.333
Electrical Conductivity (µs)	402.9	535.191	245.043	541.948
Nitrate Level (mg/L)	1.667	0	0	0
Phosphate Level (mg/L)	34	34.333	45	30.667
pH	7.708	7.795	8.27	7.777
RBP Score	118.4	124	143.4	111.4

\*(Table 1) The values of each data average are compared to the RBP. Lower temperature, nitrates, and phosphates all indicate better stream quality. High dissolved oxygen indicates better stream quality. The closer pH is to 7, and the closer EC is to 800µs, the better.



\*(Graph 1) This graph represents how the RBP’s assessment of stream quality compares to the pollution tolerance of macroinvertebrates found at the stream. The lower that the average pollution tolerance is, the better the respective stream quality.

### Conclusion

After testing each stream with both methods, analyzing the results, then comparing the results, we concluded that the RBP is efficient cost-wise, because it is free, and is also successful enough in determining the quality of streams to be a national standard, despite its subjective nature. This is a financially conservative method, but our qualitative methods, although expensive at about \$1800-\$2000 for good equipment, result in a much more accurate summation of the streams quality. The RBP doesn’t include certain aspects of stream quality which we considered important, such as the macro-invertebrate tolerance levels, though they do include a section on this with missing data for our area of study, and chemical components of the streams. When comparing the RBP component scores to our weighted averages of pollution tolerance for each stream, we have concluded that

there is not a strong correlation between them at all, indicating that the RBP doesn't focus at all on the faunal aspect of streams. Also, the RBP doesn't focus on chemical aspects of the stream and this is indicated by Table 1 which shows how the highest RBP score, and the healthiest stream by their standards, has the highest phosphate level, which is bad. Instead, a majority of the RBP's focus is based on floral and environmental aspects to assess stream quality.

During the course of our experiment, a number of questions developed over time that made us want to pursue future directions. The most interesting of the possibilities was an analysis of flora and fauna around the stream of interest and how it changes the quality of the stream. Other possible ideas for experiments we came up with were assessments of larger bodies of water under the same circumstances, an in-depth look at the relationship between pH and macro-invertebrate tolerance, and a study of the correlation between species diversity and riparian width.

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# **Fact Retention as Affected by Varied Media**

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## **Abstract**

Education, a vital necessity of today's society, must be forward-minded and continuously improved. For these reasons, constant research is being accomplished in this field of study. The most significant discovery is that of learning styles, the ways people learn and remember new facts and information. There are three major kinds of learning styles: auditory, visual, and kinesthetic. This study tested those of auditory, learning best by listening to the information, visual, learning best by seeing or watching the information, and verbal which is a combination of auditory and visual. Reading is considered to be verbal learning. These styles were tested by distributing information through video, audio recording, and print copy, and testing the three different groups. This study sought to find information beneficial to the area of education and found its hypothesis to be incorrect. Video distribution did not have the largest number of high-scoring tests. Written distribution had the largest number of students with high scores, and a higher score range of 7-11\* (*see Figure 4*) to Video's 6-10 and Auditory's 5-9.

## **Introduction**

Education, being an important aspect of today's society, has many a time undergone change as scientists and educational researchers find new ways to improve it. One of the most studied aspects of education is learning styles which are, as defined by Dr. Rita Dunn, "the ways individual people begin to concentrate, process, internalize, and

remember new and difficult material.” There are four major categories of learning styles, visual, auditory, kinesthetic and tactile (Koch, 2007). Visual learners study best when using visual aids, such as maps, diagrams, and videos. Auditory learners study best by listening to lectures, watching videos, and participating in group discussions. Kinesthetic and tactile learners do best when being active in activities such as science labs, field trips, and playing memory games (Fleming, Learning Styles). Studies under Dr. Rita Dunn, at St. John’s University were attempting to help teachers change their teaching methods to fit those of different types of learning styles by changing the way they distribute information so a student can more easily understand. This study will seek to find a correlation between fact retention and media and if changing a teaching method is really necessary. Will more facts be retained by watching a video of selected material, listening to an audio recording of selected material, or reading a print copy of selected material? Does the way information is distributed really matter? This research will try to answer these questions by giving high school students, ages 14-18, a ten question quiz over “Scene 14” of David Mamet’s *A Life in the Theatre*.

### **Methods and Materials**

This study required a video camera, voice recorder, script, copy machine, and tested 432 high school students ages 14 to 18. Before testing began, “Scene 14” of David Mamet’s *A Life in the Theatre* was selected as the information for distribution. This play was chosen because it required only two actors, which made video production easier and faster. “Scene 14” was chosen from the play because it had the lowest risk of upsetting any student. Once the desired information for distribution was selected, a video was made of “Scene 14” using a video camera. Following video production, a voice recording was

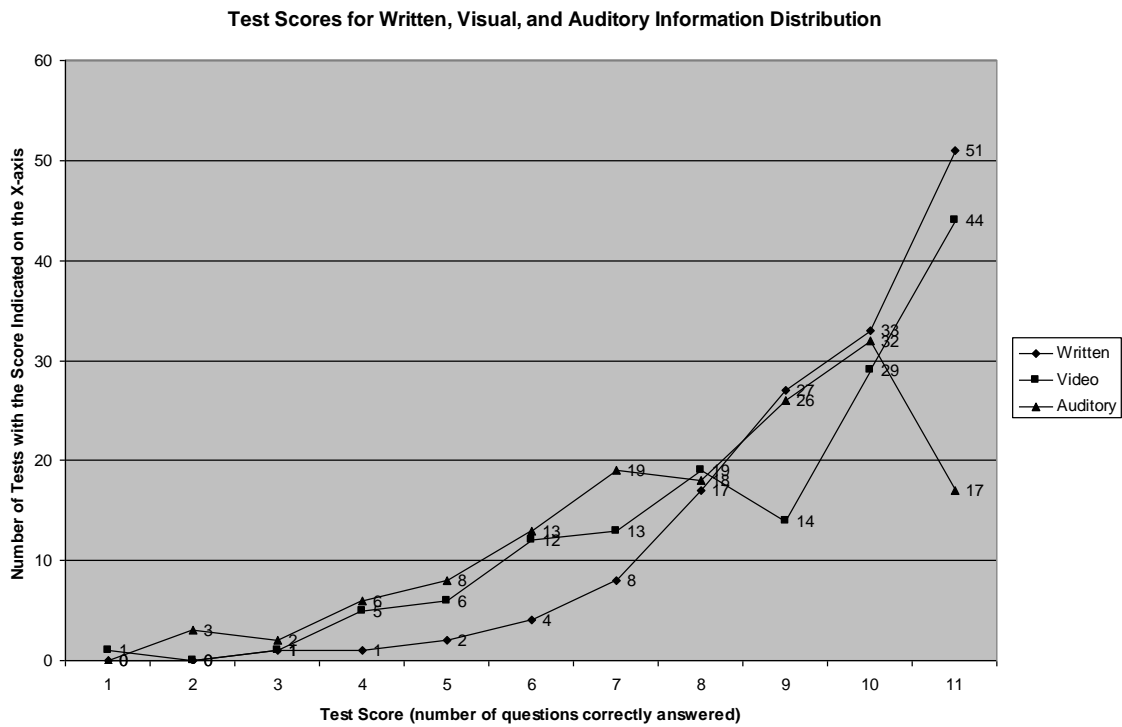
made of the material using a voice recorder and the same actors from the video. Once an audio recording and video recording were made, 105 copies of the script were printed using a copy machine. After the video recording, audio recording, and print copy were ready for distribution, a ten question quiz was written to test the students on the information contained within the video recording, audio recording, and print copy of “Scene 14.” The high school chosen for the study was chosen for its easy access to the research. Since English teachers teach all students in the school, their assistance was used in getting volunteers. Once the research was prepared for information distribution, the nine English teachers within the high school were asked for their assistance. Once all nine agreed, three of the teachers were given print copies of “Scene 14,” (*see appendix*) three were given video recordings of “Scene 14,” and three were given an audio recording of “Scene 14.” Each English teacher was also given a letter outlining the direction and containing a copy of the consent form to read to their students, 35 copies of the quiz, and 35 answer keys (*see appendix*). The English teachers were also asked to have their students refrain from putting their names on their answer sheets. Out of the almost 1000 students asked, 432 volunteers gave usable answer sheets. Usable answer sheets were those that came from volunteers and were void of any names. English teachers were given one week to ask students for their participation and administer tests to the students who were willing to participate in this study. Results were collected every day after school directly from the English teacher after the teacher was asked to double-check for named answer sheets. If any named sheets were found, they were removed and disposed of by the teacher and were in no way collected. Once applicable answer sheets were collected, they were taken to the home of the researcher, graded by the researcher, and kept at the



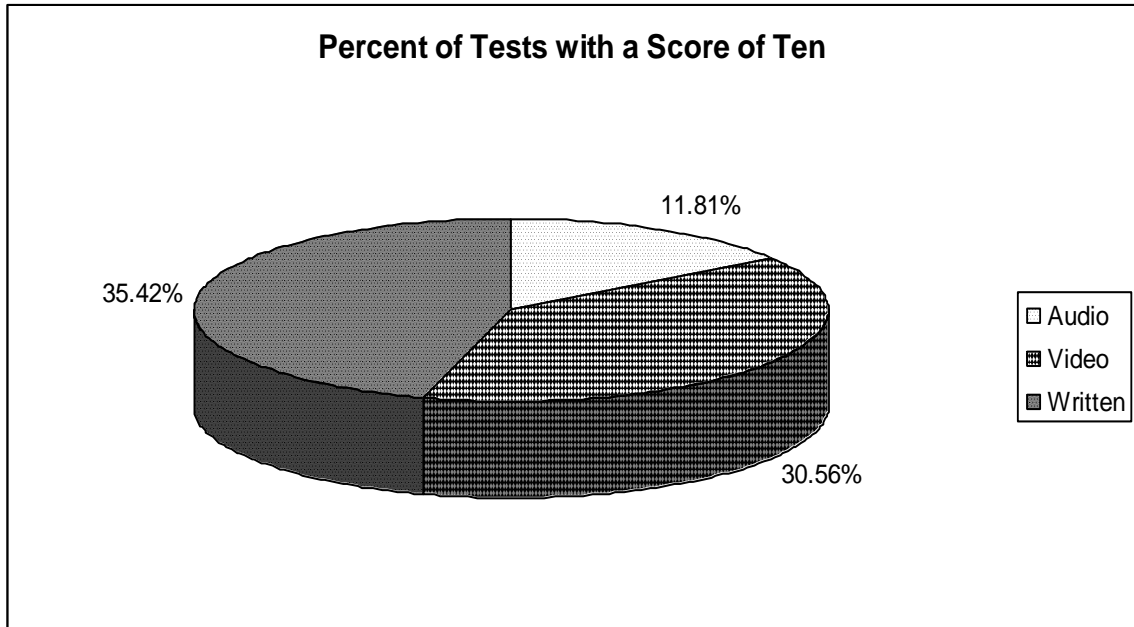
home of the researcher. The grade put on each paper was a plus sign followed by the number of answers correct. Blanks were considered incorrect since the quiz was testing memory and a blank was seen as a lapse in fact retention and recall. Quizzes with equal scores were grouped together within their own category: video, audio, or print. Scores were then tallied and put into charts.

## Results

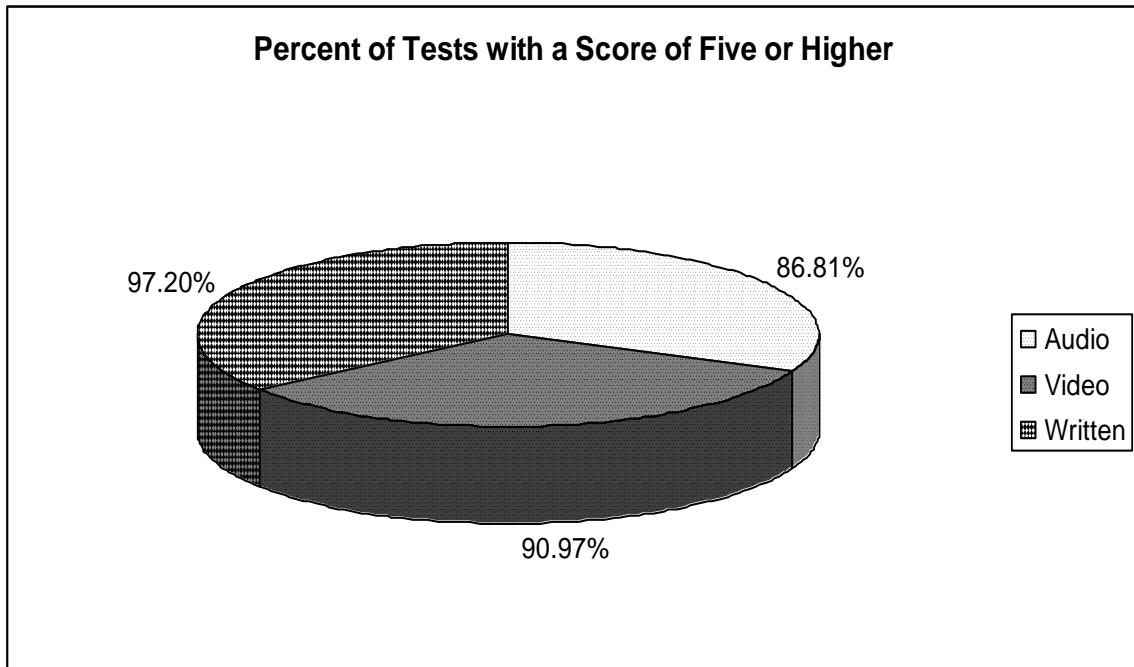
**Figure 1**



**Figure 2**



**Figure 3**



**Figure 4**

*\*A ten was the highest possible score to achieve.*

Learning Style	# of Subjects	Mean	Standard Deviation	Range
Auditory	144	7.1	2.2	5-9
Video	144	7.8	2.2	6-10
Written	144	8.5	1.7	7-11*

## Conclusion

Eleven point eighty-one percent (17 out of 144 tests) of subjects who listened to the audio recording correctly answered all ten questions on the test, scoring a perfect ten. Also 75.00% (108 out of 144 tests) of subjects scored between a five and nine; this means they remembered as many as or more than five facts out of the ten tested. Thirty point fifty-six percent (44 out of 144 tests) of subjects who watched the video representation of the tested material remembered all ten facts they were asked to recall. Sixty point forty-two percent (87 out of 144 tests) scored between five and nine. Thirty-five point forty-two percent (51 out 144 tests) who read the tested material scored a ten on the test, and 61.81% (89 out 144) scored between a five and nine. The results of this study contradict the hypothesis that the majority of high scores would be achieved by the subjects that viewed the video of the script.

Collected data illustrate that the subjects who read a printed copy of the script had the largest number of high scores which suggests print to be the best form of information distribution. However, this is only on a short-time basis, the amount of facts immediately recalled after exposure to the desired information, not a long-time basis which would give the students time to study and memorize desired information. This also suggests that in a short time, most students are verbal learners, a combination of auditory and visual. This insight is beneficial to teachers as this and further studies may help them change their teaching methods to more effectively educate children. In order to find more reliable results that may better assist teachers with such a goal, improvements should be made to this experiment. Such improvements include bubble answers to prevent handwriting identification and answer choices such as “I can’t remember” and “I choose not to

answer” to discern between lapses in memory and his or her right to refuse to answer a question. Interesting additions to this study for future research are sorting the media according the students’ learning styles to see if scores will be higher and making it a long term study to scores will be higher.

## Appendix

Dear English teacher,

Thank you for assisting me in this research study. Along with these directions you will find 35 copies of the test and an answer sheet. The 35 tests are of course a class set, so it is important that the students use their own paper, and the answer sheet is only there to give you any possible information you may need. You do not have to check the students' tests for correct answers. The following directions should be followed as much as possible, but first, read the following consent form to your students. **It must be read to each English class before the study begins.**

Today I am going to use about fifteen minutes of class time to help one of our students with her science fair project. She wants to see if the way a small portion of a play is presented (by video, audio recording or printed page) will impact how much students can remember about what happened in the play.

It will take about 15-20 minutes to be a part of this study. After a short part of the play, you will be asked to answer 10 questions about it. Whether you choose to participate or not will not affect your grade in this course and if you decide to do it and change your mind, that is okay too. You may also skip a question if you choose.

Finally, you are asked not to put your name on the paper so that your answers will be private. You may skip a question if you choose.

- 1.) Distribute the information.
- 2.) Impress upon student's that they should NOT write their names on their answer sheets. I cannot accept answer sheets with identifiable information. If you would be so kind as to discard any answer sheets that have identifiable information, I would greatly appreciate it. I CANNOT accept answer sheets with identifiable information.
- 3.) Take note of any outlying circumstances that may skew results such as a fire drill.
- 4.) Do not allow the students to view the video a second time or listen to the recording a second time. If your students are reading the material, it is your choice as to what is the best method of regulating time so students have relatively equal time to absorb the information.

Thank you again for all of your help, and I will pick up results afterschool. Good luck with testing.

Respectfully,  
Elizabeth Coleman

## Scene 14

*Robert and John are eating Chinese food at the makeup table between shows.*

Robert: You had an audition this afternoon, eh?

John: Yes.

Robert: how did it go?

John: Well, I thought.

Robert: Yes?

*Pause*

John: They were receptive. I thought it went well.

Robert: How did you feel?

John: I felt good; they liked it.

Robert: That's nice.

John: I thought so.

Robert: That's very nice. (*Pause. Eating*) There are two classes of phenomena.

John: There are.

*Pause.*

Robert: There are those things we can control and those things which we cannot.

John: Mmm.

Robert: You can't control what someone thinks of you.

John: No.

Robert: That is up to them. They may be glum, they may be out-of-sorts. Perhaps they are neurotic.

John: How's your duck?

Robert: Fine. (*Pause.*) One *can* control, however, one's actions. One's intentions.

John: Pass the bread, please.

Robert: That is all one can control.

John: Please pass the bread.

Robert: You're eating bread?

John: Yes.

Robert: Oh. (*Pause.*) Here it is.

John: Thanks.

Robert: If they hadn't liked you, that would not have signified you weren't a good actor.

John: No. I think I know that.

Robert: Yes. I think perhaps you do. *(Pause.)* Yes. I'm glad they liked you , though.

John: Thank you.

Robert: You think they are going to hire you?

John: I don't know.

Robert: Well, I hope they do.

John: I hope so, too.

Robert: That would be nice for you.

John: Yes.

*(Pause.)*

Robert: *(to self)* Good things for good folk.



Quiz: David Mamet's A Life in the Theatre  
Scene 14

- 1.) What are the names of the two Characters?
- 2.) What are they eating?
- 3.) Of what profession are the two men?
- 4.) What are they discussing?
- 5.) Who went to the audition?
- 6.) Who is eating duck?
- 7.) Who wants bread?
- 8.) Where are the two men sitting?
- 9.) Does John want to be hired?
- 10.) According to Robert, can you control what someone thinks of you?

## Quiz Answer Key

Answers can include any of the following and still be correct.

- 1.) Robert and John-(this answer must have both names to be correct.)
- 2.) Chinese food, duck, bread
- 3.) Acting, actors
- 4.) John's audition, what people think
- 5.) John
- 6.) Robert
- 7.) John
- 8.) Dressing room, backstage, makeup table
- 9.) Yes
- 10.) No

## **Acknowledgements**

This study would not have been possible without the assistance of many people. Thanks must be given to the English teachers of Northwest High School: Penny Blane, Dawn Burgess, Rachel Knapp, Cresta McGowan, Shirley Ross, Jill Sleigh, Laurie Stansbury, Michael Watson, and Sandra Webb for their agreement to ask their English classes for volunteers, Constance Brown for her encouragement to pursue this study topic, Dr. Robertson for his suggestion of using drama, and Dr. Nanci Woods for her assistance through the preapproval process. Thanks also go to MTSEF and TJAS for presenting me with the opportunity to share my work. Thank you to Emily Travis and Ethan Bisgaard for teaching me the ways of Microsoft Excel. Thank you to Tyler Tidmore and Joe Walsh for acting in the video, and thank you to the Roxy Regional Theater for providing its facility to film the video.

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<<http://www.auburn.edu/~witteje/ilsrj/Journal%20Volumes/Vol1me1Fall2007%20PDFs/Conversation%20with%20Dr.%20Rita%20Dunn.pdf>>.

# **How Does the Absence of Light Affect the Biological Clocks of Common Tennessee Crickets?**

Rezzan Hekmat, Rita E. Pfeiffer, Anh Pham  
School for Science and Math at Vanderbilt, Nashville

## **Abstract**

Exposure to light governs the biological clocks of most organisms. We tested the effects of a constant dark environment on the circadian cycles of the common Tennessee cricket. Two crickets in each group were placed in activity monitoring wheels. We found that the L:D crickets' onsets and offsets progressively started earlier in the day. The D:D crickets' onsets and offsets were relatively constant, and thus, their hours of activity were relatively constant. Our data showed that the L:D cycle crickets displayed less synchronized activity levels, while the D:D cycle crickets displayed a more synchronized pattern.

## **Introduction**

In every sound body, there is a sound mind. Not only for humans but also for most organisms, sleep is crucial for mental and physical rest (Kumar, 2008). With this comes the common conception that in order for people to lead a healthy lifestyle, they must eat three times a day and should sleep at least for 6 hours (Kumar, 2008). But how exactly do systems know when to do this?

The answer is circadian rhythms, which are biological clocks. "Clock genes" that bear the genetic instructions to generate proteins manage these rhythms (Siegel, 2009). The rhythmic patterns are based upon the fluctuations in the levels of these proteins (Siegel, 2009). The circadian rhythms control various functions: everything from locomotor activity cycles to

temperature levels, heart activity, hormone secretion, blood pressure, oxygen consumption, and metabolism (Siegel, 2009).

The circadian clock in organisms is important for evolutionary fitness in that they regulate many bodily functions (Emerson, Bradshaw, and Holzapfel, 2008). In an experiment with *Wyeomyia smithii*, a pitcher plant mosquito, larvae were synchronized into diapause and then put under different light conditions (Emerson, Bradshaw, and Holzapfel, 2008). Then the mosquitoes' fitness was calculated based on the per-capita expectation of future offspring, fecundity, embryonic viability, and adult longevity (Emerson, Bradshaw, and Holzapfel, 2008). They found that although pupal survivorship, embryonic viability, and adult longevity were unaffected by the variable of light, reduction in fecundity lowers fitness in a nonresonant L:D environment (Emerson, Bradshaw, and Holzapfel, 2008). The experiment was able to support the idea that an internal clock is adaptive in natural populations and that being in concordance with the environment's cycle would maximize fitness (Emerson, Bradshaw, and Holzapfel, 2008).

Light is commonly used to synchronize circadian rhythms (Emerson, Bradshaw, and Holzapfel, 2008). As some organisms live in areas of minimal light exposure, we were curious as to how the absence of light affects these clocks and the period changes that result from this. Our model organism, a common Tennessee cricket, was used as a model for future research on the cave cricket, *Hadenoeus subterraneus*, which is accustomed to living in a completely dark environment (D:D). Crickets in many environments create the basis of the food chain (Johnson, 2008). Understanding these foundations is crucial for the conservation of karst ecosystems (Johnson, 2008); once the bottom collapses, the food web stumbles over as well. We hypothesized that the crickets not exposed to light (D:D) would have a random cycle over time,

whereas crickets in a controlled, 12 hour light to 12 hour dark cycle (L:D) environment will have a more synchronized and consistent cycle.

### **Methods**

In preparation for housing our model organism, we created a climate-controlled and light-isolated environment. We divided an enclosed area into two equal sections using hard foam insulation in order to have two test groups: a D:D group and a L:D group. In order to simulate the light cycle in the L:D group, we used one compound fluorescent light as the light source. Connected to a light timer, this compound light would turn on at 600 hours and turn off at 1800 hours, creating 12 hours of light and 12 hours of darkness. In addition, we used the hard foam insulation to cover any openings in the enclosure, allowing us to control both the temperature and light in the study environment. Within these large sectional environments, we inserted one reptile tank to contain surplus crickets.

Our test subjects, the common Tennessee crickets, are indigenous to the western savannah of Tennessee. After obtaining 24 crickets, we brought them to the laboratory. We divided them into two groups of twelve, and assigned one group to the D:D treatment and one group to the L:D treatment. Any group assigned to a particular light setting stayed in this setting for the entirety of the experiment.

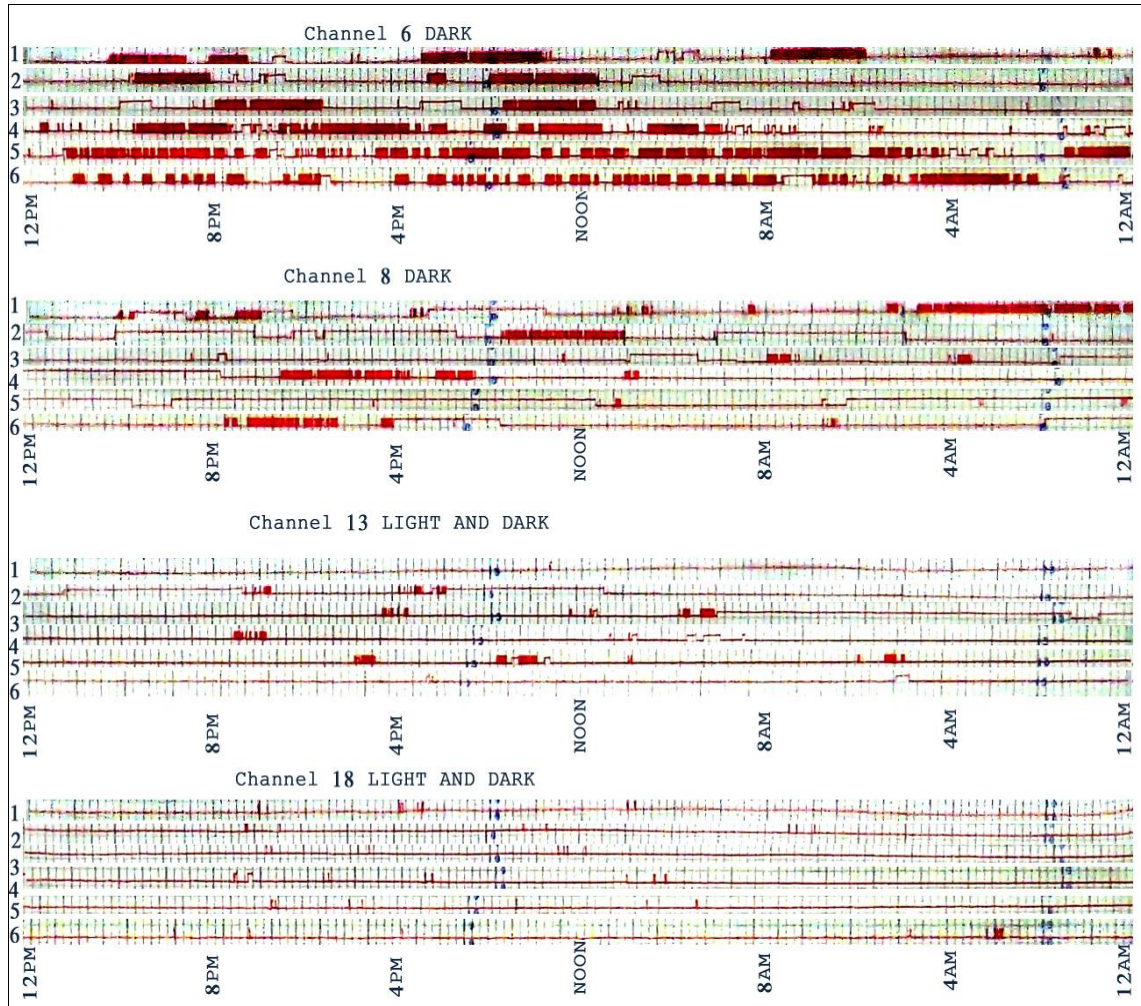
To monitor activity cycles, we used activity-monitoring wheels, which were connected to a data logger. Ultimately, the data logger recorded the activity cycles of these crickets, marking each time a full rotation was made by the crickets in the wheels. Two wheels were assigned to each environment, in which two out of the twelve crickets in each environment ran in their own separate wheel for the designated time period of seven days, and their activity was analyzed for the last six days.

## Results

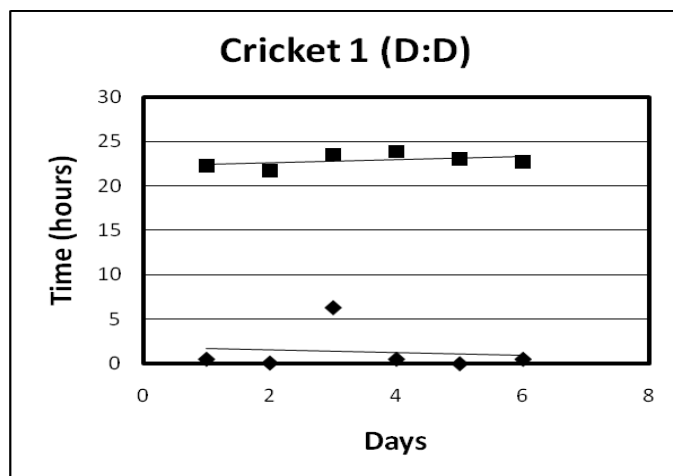
Using the data sheets obtained from the data logger, we calculated the first onset and offset of each cricket for each day of activity. The onset is the start of activity, whereas the offset is the end of activity. To calculate this, we found the time when the overall activity first began for the cricket in a given day. In that same day, we found when the activity ended. Activity was defined as completing two full rotations in the activity monitoring wheels within 10 minutes.

Seen in Figures 2a through 2d.2, the data points gained from Figure 1 were used to determine the lines of best fit for both the onsets and offsets, indicating the hours of activity for the crickets. The more parallel these lines of best-fit are to the x-axis, the more synchronized the organism. Due to the outlier on day six on cricket two of the L:D environment (Figure 2d), we created another chart in order to document the activity for this cricket without the onset and offset of activity for day six (See Figure 2d.2) in order to better depict the activity cycle for this cricket.

In addition to the activity date, we made many anecdotal observations regulated to the behavior of the crickets. We found that many of the test subjects were egg-producing females, and as a result, their clocks may have been on a different pattern than their male counter parts. In addition, the crickets living in the D:D environment were much more active than those under the L:D environment. Although crickets in both environments engaged in cannibalism, we made the intriguing observation that there were more instances of cannibalism in the L:D environment than the D:D environment. This could be a sign that the crickets in the L:D environment had more stress with light.



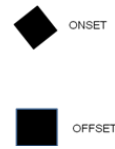
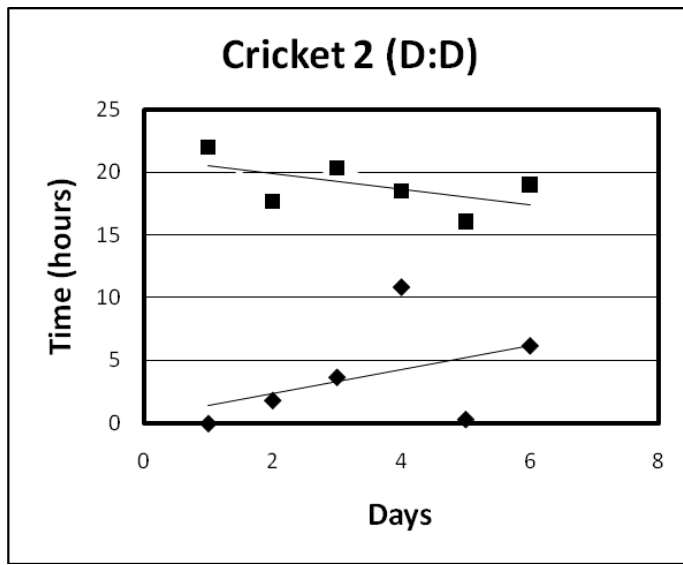
**Figure 1:** The black blocks featured above signify activity and was recorded from our data logger based on the rotations of the activity monitoring wheels made by our test subjects. The days are signified by each row, labeled 1-6. Channel 6 is D:D Cricket 1, Channel 8 is D:D Cricket 2, Channel 13 is L:D Cricket 1, and Channel 18 is L:D Cricket 2. From this, we were able to calculate the first onset and offset of each cricket for each day of analysis (seen in figures 2a-2d.2).



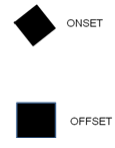
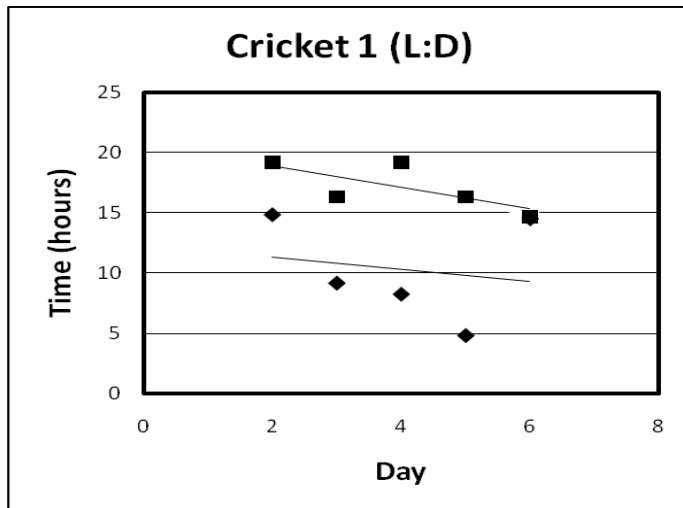
◆ ONSET  
■ OFFSET

**Figure 2a** shows the test subject in the D:D environment. This cricket had a relatively stable cycle from start to finish as the activity hours stayed constant. Hours of activity are shown in between the onset and the offset lines of best fit.

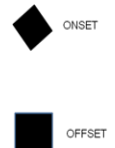
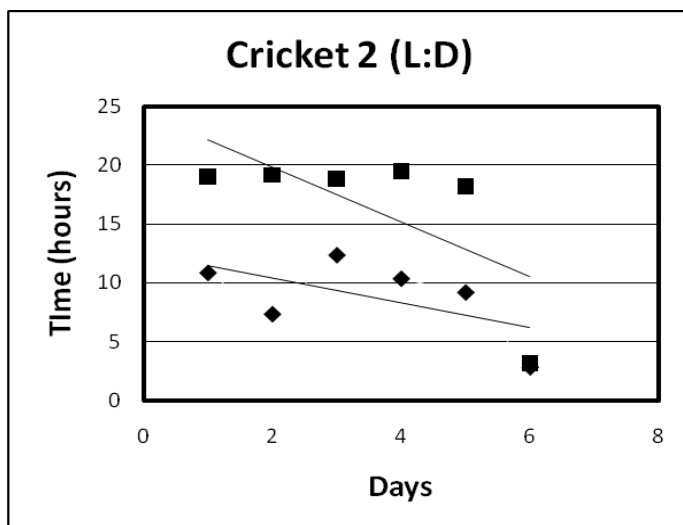




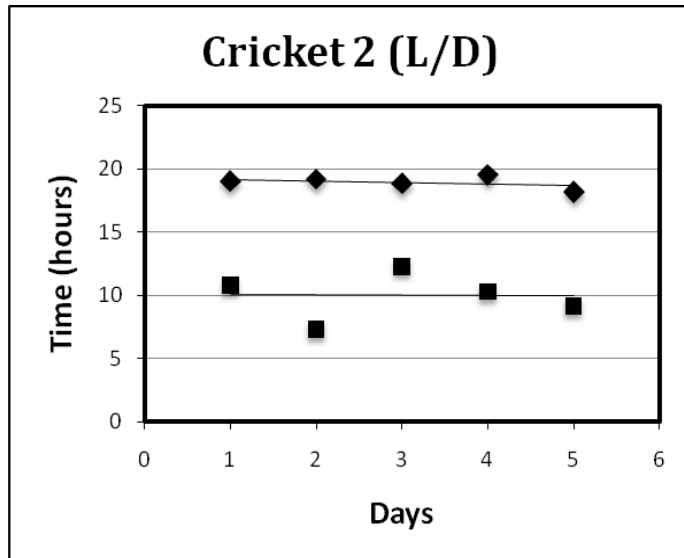
**Figure 2b** displays another test subject in the D:D environment. Unlike Cricket 1 (D:D), the hours of activity changed as the days progressed. In this case, Cricket 2 (D:D) had fewer hours of activity on Day 6 than in Day 1, meaning that the onsets started earlier and the offsets started later on in the day.



**Figure 2c** shows Cricket One (L:D) during the six day cycle period. While those in the D:D environment had a relatively stable period, this cricket experienced more changes in its cycle. The hours in which the crickets were active stayed relatively the same, but their days started earlier in each day, signaling an unsynchronized cycle.



**Figure 2d** had similar results compared to Figure 2c. However, what changed were the hours of activity and the degree of change seen in this cricket. The cricket had drastically decreased their hours of activity by nearly half by day six, and started their days earlier by approximately 5 hours.



**Figure 2d.2** shows the results of the activity cycles of Cricket 2 in the L:D environment without the outliers on day 6. This graph shows that this cricket had a stable activity cycle.

### Conclusion

Over time, the crickets in the L: D environment had a more random cycle than those in the all dark environment, indicating our hypothesis incorrect. Our results showed that the presence of light does have a great effect on the activity levels of these crickets. When exposed to light, the crickets, which normally live in total darkness for extended periods, eventually functioned with a free-running rhythm with their activity cycle being pushed back or forward each day. However, when kept in total darkness the activity of the crickets became more synchronized, changing little over time. In addition, an anomaly that we observed was that there was a higher rate of cannibalism in the L:D environment than the D:D environment. The crickets seem to incur greater stress under extended periods of light exposure. To further our research, this same species of cricket will be used again, only there will be more crickets used in each light condition for a more extended period of time. This future research proposal is also to verify the results seen in Figure 2d.2, as this graph seemed peculiar to our research group.

Although light is one of the major phasing factors, there are more environmental cues that are capable of setting the period of a biological rhythm, or Zeitgeber (Lu, No Date). Other

major Zeitgebers of biological clocks are composed of a number of abiotic factors such as temperature and humidity cycles (Sharma and Chandrashekar, 2005). In addition, there are different biotic factors that can contribute to the synchronization of these biological clocks, such as inter-individual interactions and interactions with prey, predators, and parasites (Sharma, Chandrashekar, 2005).

The absence of light typically has staggering effects on different organisms (Emerson, Bradshaw, and Holzapfel, 2008). Without light to synchronize the circadian clocks of these organisms, such as humans (Kumar, 2008), they have a hard time adjusting. Light, an environmental cue (Sharma, Chandrashekar, 2005), led us to question organisms that do not have access to it, yet are still able to function perfectly well. Cave crickets, such as the Northeastern cave cricket, *Hadenoeus subterraneus*, stay in the caves for almost two weeks until they leave for food (Hubbel, Norton, 1978). Even when the crickets are not in the cave, they only leave on sufficiently warm and moist nights (Hubbel, Norton, 1978). These crickets get even less light than blind organisms. Although many of the species that are blind lack image forming-eyes, they still have functioning photoreceptors in the rest of their body (Heimonen, Salmela, Kontiokari, et al., 2006). These photoreceptors, in turn, are what capture the light signals and send these signals to the internal clocks where they will be used for synchronizing (Heimonen, Salmela, Kontiokari, et al., 2006). The cave crickets lack the light, not the ability to synchronize. Having visited Mammoth Cave National Park, we became very interested in *Hadenoeus subterraneus*, a cave cricket common in the Eastern United States (Hubbel, Norton, 1978). For future research, we are curious to know what exactly sets the biological clocks of these crickets. If not light, than what abiotic or biotic cues have major affects on their clocks?

The circadian clock has critical affects on organisms, and for most it is the key for these biological clocks to function at their best level (Emerson, Bradshaw, and Holzapfel, 2008). However, not all organisms depend on light to synchronize (Sharma, Chandrashekar, 2005), and some can do better without it. One of these organisms is the cricket. Making up the basic foundations of the food web, the cave cricket is crucial for the stability of the karst environment (Johnson, 2008). However, recently these environments have become more and more endangered (Robins 2007). Understanding certain key elements in the environment, such as the cricket, would be crucial in the conservation of these caves (Robins, 2007).

### **Acknowledgements**

Thanks to Anh Pham, who helped us complete this research. Thanks to Dr. Glenn McCombs, our advisor, and thanks to Dr. Terry Page for providing us with our equipment and advice. Thanks to Dr. Kimberlie McCue, Dr. Angela Eeds, Dr. Chris Vanags, and Dr. Jonathan Creamer for their support and guidance

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## **ABSTRACTS OF PAPERS**

Presented at the Annual Meeting  
Belmont University  
Nashville, Tennessee  
April 17, 2010

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### **Relationships and Effects of Sedimentation and Channelization of Mouse Creek**

(Bradley County, Tennessee)

Lillie K. Brown  
Cleveland High School, Cleveland

#### **Abstract**

Measurements were taken at two sites on Mouse Creek, located in the Ridge and Valley Dolomite Ecoregion, within three habitats: run, riffle, pool. Water depths, sediment depth, water clarity, and stream width were measured. Observations were also made about surrounding environment. Sites were characterized urban and secluded due to surrounding conditions, but both were part of an urban stream. Results showed differences between the two sites in stream width, water clarity, sediment depth, and water depth. Correlations were significant between thalweg depth-stream width, water clarity-sediment depth, thalweg depth-sediment depth at both sites as well. This could possibly suggest the differences are because of the continuous urbanizing growth surrounding the watershed.

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### **The Effect of Temperature, Concentration, and pH on Lactase Efficiency**

Christine Choo  
Siegel High School, Murfreesboro

#### **Abstract**

Lactase is a digestive enzyme that breaks down lactose to produce glucose. Temperature, concentration, and pH variables were tested to observe their effects on lactase efficiency. Lactase was added to milk kept at different temperatures: 200°F, 65°F, and 40°F to test for temperature effect on enzyme activity. For enzyme concentration tests, three different

concentrations, one Lactaid tablet, two Lactaid tablets, and three Lactaid tablets were tested in milk. Enzyme efficiency was tested in acidic, basic, and neutral conditions by adding lemon juice, hydrogen peroxide, and water to milk. Enzyme activity for the first test was measured at one, three, and five minutes; in the following two, tests were measured at two minutes, four minutes, and six minutes. The results suggest that the higher the temperature, and the more time allotted for the lactase to mix with the milk, the more lactase efficient the solution would be. The results also suggest the higher the concentration of lactase tablets and the more time given for the reaction to elapse, the more lactase efficient. Therefore, the higher the temperature, concentration, and pH level, the greater the lactose breakdown.

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## **Crystallization with Vibrations**

Della Coleman  
Northwest High School, Clarksville

### **Abstract**

Sound is a major part of our world whether it's for communication or is present due to excess of motion. All sounds, even ones inaudible to the human ear contribute greatly to the formation of matter, and more specifically, crystals. Any disturbance that occurs during the growth and nucleation of a crystal determines the final structure produced. Delving into this fact, the presented experiment targets the question of how sound frequency affects crystallization. During this study an A<sub>3</sub> pitch of 440 Hz was applied to a crystallizing sugar mixture of the same composition crystallized naturally. It was hypothesized that the added vibrations would either weaken or strengthen the crystalline structure. The experiment provides insight into the field of crystallography and physics.

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## **Aquatic Ecotoxicological Test Using Planaria**

Ryan Kilpatrick & Thomas Griffin  
Pope John Paul II High School, Hendersonville

### **Abstract**

Chemicals which we use and carelessly dispose of not only affect environments, but also influence its inhabitants. Ammonium Nitrate (NH<sub>4</sub>NO<sub>3</sub>) was used to create scenarios of varying

acidity. Planarian have the unique ability to regenerate lost body parts. In this experiment, the success rate of the planarian regeneration was used to measure acidity. Once the experiment began, the flatworms were cut in half, and the rate at which they regenerated was measured and compared between four different concentrations. At the conclusion of around one week, all of the planarian halves had either regenerated or died. Analysis of these findings was then in order. The result of this experiment is a better understanding of planarian regeneration and ecotoxicology.

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## **Factors That Influence the Decomposition Process**

Jessica Huang  
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### **Abstract**

During composting, several factors can determine how effectively a substance decomposes, a few being heat, carbon to nitrogen ratios, and content. These can be tested by comparing the masses of trials under the different conditions of temperature, carbon to nitrogen ratios, and the presence of supplementary materials. The outcome after testing this was that the trial under heat, with a 1:3(C:N) ratio, and additions of manure and starter decomposed the best. The heat trials did significantly better than those under no heat (p value 0.00183), and the group under no heat also had a statistical difference from their beginning mass (p value 0.00158). Originally, the best results were assumed to come from boxes with 3:1 carbon to nitrogen ratios, those containing manure and/or starter, and those under constant heat, but instead, the ratios ended up being different. The 1:3 (C:N) ratio was the most effective because of unusual levels of carbon in the materials used, and thus the higher amount of nitrogen substances nullified the unusual concentration of carbon. Composting is an important process in nature, and using the different factors to their greatest advantage while composting biodegradable substances could help the environment by limiting landfills and reusing organic materials.

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## **Effects of Age on Habituation in Mice**

Thomas Koen  
Pope John Paul II High School, Hendersonville

### **Abstract**

The experiment dealt with the way increased age effects the time it takes for a rat to habituate to a novel stimuli. It was predicted that, as age increased, habituation time would decrease. Using two age groups with four test subjects each, the procedure used high frequency sound in combination with the lure of food to create a situation where the rat wanted to eat but, had to overcome the instinctual reaction to stay away from a stimulus to do so. Eight rats, four young and four old, were subjected to a test that measured the time it took to adjust to a novel stimuli. Each rat was placed in a container with food in one end, and each time the rat would approach the food, a high-pitched sound would be played until the rat either turned away or took the food away. From the data gathered, the older rats habituated after an average of 3.5 exposures. When the data was subjected to a one-way ANOVA analysis, the p value was 0.211, thus showing that the data was both statistically relevant and in support of the original hypothesis.

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## **Nest Predation in the Murfreesboro Flat Rock Cedar Glades Using Height and Color Variables**

Caitlin Levi & Nealy Pistole  
Siegel High School, Murfreesboro

### **Abstract**

Do color and height variables affect nest predation in flat rock areas? Cedar Glade areas have distinct vegetation; containing dry soil, rock such as limestone, and harsh plant life. Nest predation in these areas is more prevalent due to the environment being so bare, creating an unprotected area for nests majority of the time. Blue and white clay was used for the color variable, and high and low placements were used for the height. The results showed that there was not a discrepancy amongst the variables but the data found was intriguing. The blue eggs showed a large amount of predation compared to the white eggs. This could mean several things, such as the nests containing blue eggs in the wild tend to be less protected than the white.

With the height variable, the data did show variation and had more of a preference for the higher nests. Therefore, this could mean that more avian predators attacked the nests because these predators would be able to see the eggs from over head. Due to a snow that happened, the data is a bit skewed merely because trends of animals search for food rises with the particular weather condition.

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### **Determining Potential Insect Pollinators of *Viola papilionacea* (Common violet)**

Hannah R. Asbell, Yi-Ting Huang, Katherine Roland  
School for Science and Math at Vanderbilt, Nashville

#### **Abstract**

In order to preserve a species, the reproduction process must be fully understood. Insect pollination is one of the most efficient ways by which plants are pollinated. We examined insect orders for *Viola papilionacea* pollen to identify putative pollinators. We swabbed field gathered insects to collect and observe pollen the specimens were carrying. These were compared to violet pollen samples. Of fifty-nine (59) specimen, forty-three (43) carried pollen, and twenty-three (23) carried what was probable violet pollen. By comparing insect groups, we concluded that spiders were the most probable pollinators.

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### **Effects of Habituation Rate on *C. elegans* Knockouts Using Mechanical and Heat Stimuli**

Kevin A. Roman & Aziza M. Hart  
School for Science and Math at Vanderbilt, Nashville

#### **Abstract**

We tested the locomotion of *C. elegans* mutants. For the mechanical stimulus we used eyelash brushes and tapped the worms until habituation occurred, waited one minute and repeated the previous step. In the heat stimulus we heated the platinum brush in the ethanol burner for a second, waited, and then placed it near the worms and conditioned for three minutes or until habituation. Then we waited one minute and repeated conditioning. The results of both

experiments were different in that the heat stimulus habituated faster overall. Our experiment showed that the heat stimulus had more neurotransmitters involved than the mechanical stimuli.

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## **How the Growth Rates of *Psaseolus lunatus* and *Zea mays var ravgosa* are Affected by Different Inorganic and Organic Fertilizers**

Stephanie Surgener & Caroline Elbaum  
Siegel High School, Murfreesboro

### **Abstract**

The purpose of this study is to determine if inorganic fertilizer or organic fertilizer has different effects on the growth rates of plants. This study is determining which way is the best in the fastest way of growing plants. *Psaseolus lunatus* and *Zea mays var raugosa* were used because they are the easiest and fastest growing plants. Different nitrogen concentrations of inorganic soil were used to see if the different concentrations had an effect on plant growth. Plants were also grown in organic fertilizer. For control plants, only soil with no additional fertilizer was used. After thirty-six (36) days of growth, final measurements of plant growth were taken for all the plants. Plants grown in organic soil exhibited the most seed germination and plant growth. Plants grown using inorganic showed little to no growth. Control group plants showed plant growth but not as much as the organic fertilizer. Plants grown in organic soil showed greater overall growth rates than plants grown in inorganic soil and plants grown in soil with no added fertilizer. Organic soil was best for plant growth because of the nutrients in the soil.

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## **Algae Tolerance in the Hiwassee River**

Hannah J. Johnson & Felicity H. Swafford  
Cleveland High School, Cleveland

### **Abstract**

The Hiwassee River is located within three different states; this particular study was conducted in the Southern Limestone/Dolomite Valleys and Low Rolling Hills Region of the Hiwassee River. The hypothesis of the study was to find if algae do indeed have its own tolerance levels. Six sites were chosen less than a mile apart and algae samples were taken. The

algae was identified and tested to determine its tolerance to changes in pH and temperature. All samples were found to be in the Chrysophyta and Chlorophyta algae phyla, and then were further identified into their separate genres. Each genus was found to have its own tolerance to freezing and boiling temperatures, as well as to highly acidic and basic pHs. Most algae types seemed to prefer basic environments over acidic ones, and the reaction to the temperatures seemed dependent on the algae type. There seemed to be a correlation between algae amount and shade coverage as well, though this too changed depending on the type of algae.

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## **Nursing Nuances: Juvenile to Geriatric and Gender**

Emily Travis

Northwest High School, Clarksville

### **Abstract**

Developing attitudes towards people is human nature. Negative and positive attitudes towards nurses are commonly seen among Americans of both genders, ranging from juveniles to geriatrics. Therefore, the purpose of this experiment was to determine if there was a difference in attitudes towards nurses and the nursing field in Montgomery County and the surrounding areas. An eleven-question questionnaire was given to one-hundred fifty volunteers of both genders, ages fourteen to sixty-seven. After the questionnaires were analyzed, using tables and graphs, an obvious difference in attitude and/or perspectives was present. For example, adult females indicated that they would be more pleased than teenage males if their son/daughter wished to become a registered nurse. The gathered data could be beneficial to health care workers that wish to prevent negative attitudes towards nurses and the nursing field by targeting specific age ranges corresponding to gender.

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