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98th Annual Meeting

April 27-28, 2006

Valley City, North Dakota

SCHEDULE OF PRESENTATIONS — Room 317 — VCSU Science Center

Denison Undergraduate Competition

Session 1 – Andre DeLorme - Moderator

8:40 a.m. - THE PROBLEM OF HIGH PRESSURE IN MICROWAVE ASSISTED SYNTHESIS OF NOVEL FORMAMIDE FUNGICIDES. Brittany Provencher,* Mikhail M. Bobylev, Lioudmila I. Bobyleva, Amanda C. Watts, Courtney L. Black, and Chad Dion, Division of Science-Chemistry, Minot State University

9:00 a.m. - EFFECT OF CHLOROPHENOXY HERBICIDES ON FGF EXPRESSION DURING EMBRYONIC DEVELOPMENT OF *DROSOPHILA MELANOGASTER*. Peter A. Nettleton*, Sheri Dorsam[§], Hilde E. van Gijssel, Valley City State University and [§]Department of Chemistry and Molecular Biology, North Dakota State University

9:20 a.m.- COMPUTER MODELING OF HERBICIDES IN FIBROBLAST GROWTH FACTOR RECEPTORS. Matthew J. Axtman*[1], Akash Khandelwal[2], Hilde E. van Gijssel[1]; [1]Department of Science, Toxicology Laboratory, Valley City State University and [2] Department of Pharmaceutical Sciences, North Dakota State University

9:40 a.m. - ANGIOGENIC GROWTH FACTORS EXPRESSION AND THE CULTURE AND CHARACTERIZATION OF CAPILLARY ENDOTHELIAL CELLS FROM EQUINE EXUBERANT GRANULATION TISSUE. Nicole Rowley* and Lynn Burgess, INBRE Program, Department of Natural Sciences, Dickinson State University, Dickinson, ND 58601

10:00 a.m. - THE PRESENCE OF ATRAZINE IN RIVERS AND STREAMS IN SOUTHEAST NORTH DAKOTA. Ryan Lorenz* and Andre DeLorme, Department of Science, Valley City State University

10:20 - 10:40 a.m. BREAK, Refreshments will are available on the first floor of the Science Center.

Session 2 – Andre DeLorme - Moderator

10:40 a.m. - CRANIAL DEVELOPMENT IN A MINIATURIZED FORM OF THE SALAMANDER *DESMOGNATHUS QUADRAMACULATUS*: ROLE OF TEMPERATURE AND THYROID HORMONE. Kiswendsida Claude Ouedraogo and Christopher K. Beachy, Department of Biology & Amphibian Growth Project, Minot State University

11:00 a.m. - THE EFFECTS OF LYCOPENE ON THE CELL PROLIFERATION AND EXPRESSION OF CONNEXIN PROTEINS mRNAs OF BOTH NORMAL AND CANCEROUS CELLS. Josh Seekins* and Lynn Burgess, INBRE Program, Department of Natural Sciences, Dickinson State University, Dickinson, ND 58601

11:20 a.m. - DOES SIZE REALLY MATTER? THE EFFECTS OF ATRAZINE AND COMPETITION ON GROWTH IN THE SALAMANDER (THE AXOLOTL, *Ambystoma mexicanum*) Francis Drew Henry and Christopher K. Beachy, Department of Biology & Amphibian Growth Project, Minot State University

11:40 a.,m. - PHENOTYPIC CHARACTERIZATION OF A *YERSINIA ENTEROCOLITICA FLHD* MUTANT. J. Iyer*, N. Carr, M. Townsend, B.M. Prüss, Veterinary and Microbiological Sciences, North Dakota State University

12:00 noon - NDAS Business lunch in the Skoal room of the VCSU Student Center. All registered conference participants are invited to attend.

Session 3 – Bonnie Alexander - Moderator

1:00 p.m. - CLASSIFICATION OF EMBRYO SIZE IN THE FEDERALLY LISTED THREATENED PLANT, THE WESTERN PRAIRIE FRINGED ORCHID (*PLATANTHERA PRAECLARA*). Amanda Bryson* and Bonnie Alexander, Department of Biology, Valley City State University

1:20 p.m. - DOES DNA TOPOISOMERASE II BIND IN THE *MLL* TRANSLOCATION BREAKPOINT CLUSTER REGION? Stephanie M. Mueller*, Virginia Watson, Cheryl Lepp, Kyle Pankratz, and Heidi J. Super, Department of Biology, Minot State University

1:40 p. m. - EFFECT OF CHLOROPHENOXY HERBICIDES ON THE TRACHEAL DEVELOPMENT OF *DROSOPHILA MELANOGASTER* EMBRYOS. Raymond Caylor*, Bridget Blunck, Hilde E. van Gijssel, Science Department, Toxicology Laboratory, Valley City State University

2:00 p.m. - IDENTIFICATION OF UNKNOWN MARINE CYANOBACTERIA FROM THE GENUS *SPIRULINA*. Judd E. Entzel* and Paul W. Lepp, Department of Biology, Minot Sate University

2:20 p.m. - LEAF ATTACHMENT APPEARS REQUIRED FOR INHIBITION OF LEAF EXPANSION IN ARABIDOPSIS BY THE PLANT HORMONE INDOLE-3-ACETIC ACID
Morgan L. Grundstad* and Christopher P. Keller, Department of Biology, Minot State University

2:40 p.m. - A LOWER PRESSURE PROCEDURE FOR MICROWAVE ASSISTED SYNTHESIS OF NOVEL FORMAMIDE FUNGICIDES. Ronald F. Scott*, Mikhail M. Bobylev[§], Lioudmila I. Bobyleva[§], Amanda C. Watts[§], Courtney L. Black[§], Brittany Provencher[§], Charmane F. Disrud, Tammy L. McBride, Robin R. Belgarde, and Garrett B. Mcarthur, Turtle Mountain Community College, Belcourt, North Dakota and [§]Minot State University

3:00 p.m. - ESTABLISHING INHIBITORY CONCENTRATIONS OF NOVEL FORMAMIDE FUNGICIDES ON FUNGI PERTINENT TO MEDICINE AND AGRICULTURE
Michaela M. Schwan* and Mikhail M. Bobylev, Minot State University

3:20 p.m. to 3:40 pm – Break, Refreshments will are available on the first floor of the Science Center.

Session 4 – Tom Gonnela - Moderator

3:40 p.m. - ALPHA-2A ADRENERGIC RECEPTOR-MEDIATED INHIBITION OF RAT HIPPOCAMPAL EPILEPTIFORM ACTIVITY. Jessica A. Lichter*, Chris W. Jurgens, Sarah J. Boese, Jacob D. King, Brian W. Nelson, Kylie L. Davis, Brianna L. Goldenstein, Kendell Graywater, Elisha Lawrence, James E. Porter & Van A. Doze, Department of Pharmacology, Physiology & Therapeutics, University of North Dakota

4:00 p.m. - APPLICATION OF FLUORESCENT PROBE LIFETIMES TO IMPROVE RT-PCR AND SNP DETECTION METHODOLOGIES. Morgan Gallagher*, Thomas P. Gonnella, and Khwaja Hossain, Department of Chemistry, Mayville State University-

4:20 p.m. - MOPRHOLOGY AND VASCULARIZATION OF AUTOTRANSPLANTED OVINE OVARIES AFTER FREEZING AND THAWING. Tyson S. Burkle*, Ewa Borowczyk, Jerzy J. Bilski, Jashoman Banerjee[§], Won-Jun Choi[§], Rakesh K. Sharma[§], Ashok Agarwal[§], Tommaso Falcone[§], James D. Kirsch, Kim C. Kraft, Robert M. Weigl, and Anna T. Grazul-Bilska, Department of Animal and Range Sciences, North Dakota State University and [§]Department of Obstetrics and Gynecology, The Cleveland Clinic Foundation

THE PROBLEM OF HIGH PRESSURE IN MICROWAVE ASSISTED SYNTHESIS OF NOVEL FORMAMIDE FUNGICIDES

Brittany Provencher,* Mikhail M. Bobylev, Lioudmila I. Bobyleva, Amanda C. Watts, Courtney L. Black, Chad Dion
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Microwave-assisted Leuckart reaction comprises a fast and convenient method for obtaining novel formamide fungicides. A typical reaction takes only 3 minutes, which seemingly opens the way to automated parallel synthesis of large series of analogs. However, a high pressure developing during the reaction makes it impossible to use the existing reaction vessels and equipment designed for automated microwave synthesis. Indeed, a typical synthesis of 4 mmols of a formamide fungicide analog that is carried out in 20 ml of formamide, produces 140-200 PSI pressure in an 80 ml GreenChem reaction vessel. At the same type, typical equipment for automated microwave synthesis uses 10 ml vessels which do not have enough head space to accommodate this level of pressure. In this work the factors influencing the high pressure were investigated. It was found that most of the pressure is produced not by the reaction itself but by formic acid or water that are used as additives to accelerate the reaction. Lowering the concentration of additives can reduce pressure up to two times, but will lead to lower yields and lower quality products. The results of the work suggest that the problem of high pressure in microwave assisted Leuckart reaction as well as other high pressure reactions should be specifically addressed at the hardware level.

EFFECT OF CHLOROPHENOXY HERBICIDES ON FGF EXPRESSION DURING EMBRYONIC DEVELOPMENT OF *DROSOPHILA MELANOGASTER*

Peter A. Nettleton*, Sheri Dorsam[§], Hilde E. van Gijssel
Valley City State University and [§]Department of Chemistry and Molecular Biology, North Dakota State University, Fargo, ND

Recent publications have shown an increase in the occurrence of respiratory and circulatory birth defects in wheat producing areas, especially among males conceived during the herbicide spraying season. It has been suggested that chlorophenoxy herbicides may be responsible. The chlorophenoxy herbicides, 2,4-Dichlorophenoxyacetic acid (2,4-D) and 2-Methyl-4-chlorophenoxyacetic acid (MCPA), are used extensively in wheat production. They had been considered relatively safe until they were recently reclassified as endocrine disruptors, which means that they can have significant effects at low concentrations. Our hypothesis is that chlorophenoxy herbicides cause disruptions in the fibroblast growth factor (FGF) expression, which lead to abnormal development.

The goal of our experiments, using *Drosophila melanogaster* embryos as a model system, is to determine a timeline of normal FGF expression in embryos and study the effects of embryonic exposure to chlorophenoxy herbicides on FGF expression. To determine FGF expression levels, we used real-time quantitative RT-PCR with SYBR green detection chemistry. A 1 hour embryo prelay was followed by a 30 minute lay to ensure that all experimental embryos were approximately the same age, and to reduce variation in the FGF mRNA levels. Following the pre-lay, embryos were allowed to continue development for 11, 12 and 13 hours. Subsequently, RNA was isolated from the embryos using the TriReagent method and was stored in formazol to preserve the stability of the RNA until further use. Next, RNA was precipitated out of the formazol and dissolved in water. The concentration of total RNA was measured using spectrophotometry. Thirty mg of embryos yielded 23-30 μg of total RNA.

One microgram of RNA was reverse transcribed in to cDNA and used as template for primer optimization. The set of primers for FGF chosen using IDT technologies SciTools was shown to produce only one PCR product by real-time RT-PCR and SYBR green dissociation curve analyses. Thus, these primers can be used in future studies on the expression profile of this important molecule during the first 24 hours of *Drosophila melanogaster* development.

Supported by NIH Grant P20RR016741 from the NCRR

COMPUTER MODELING OF HERBICIDES IN FIBROBLAST GROWTH FACTOR RECEPTORS

Matthew J. Axtman*[1], Akash Khandelwal[2], Hilde E. van Gijssel[1]
[1]Department of Science, Toxicology Laboratory, Valley City State University and [2] Department of Pharmaceutical Sciences, North Dakota State University.

A recent study has shown an increase in circulatory and respiratory birth defects in areas of high wheat and corn production, especially in males. The herbicides that have traditionally been used are 2,4-Dichlorophenoxyacetic acid (2,4-D), 2-methyl-4-chlorophenoxy-acetic acid (MCPA), 3,5-Dibromo-4-hydroxybenzonitrile (Bromoxnil), and 2-Chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine (Atrazine). These herbicides have been classified as endocrine disruptors, which mean they may have significant effects on hormonal pathways in low concentrations. Fibroblast Growth Factors (FGF) and FGF receptors are involved in many processes including lung development. Our hypothesis is that these herbicides will cause a significant disruption of the FGF pathways in developing embryos, which may lead to respiratory birth defects.

Our goal is to determine if 2,4-D, MCPA, Bromoxnil, and Atrazine can bind to the FGF receptors using computational approaches. The crystal structure available in the protein data bank (pdb) doesn't contain any co-crystallized inhibitors, so our first goal was to identify the binding site and then dock the herbicides into the binding pockets. The crystal structures with highest resolution for Fibroblast Growth Factor Receptor 1 (file 1FGK) and Fibroblast Growth Factor Receptor 2 (file 1GJO) were downloaded from the pdb. Three-dimensional structures of herbicides were constructed using the SYBYL7.1 suite of programs running under Irix 6.5. The binding pockets in FGFR-1 and FGFR-2 were determined using SiteID. The results of SITEID indicate that there are five different binding pockets for FGFR1 and three binding pockets for FGFR2. One of these pockets (both in FGFR-1 and FGFR-2) is within the catalytic loop, which is the active site of the receptor and most likely has the most significant effect. The herbicides were then docked into the individual pockets using FlexX. The bound conformations for each herbicide were selected based on FlexX score.

Computational approaches are excellent tools for identifying binding sites and subsequent docking studies. The results of the modeling need to be confirmed by doing an *in vitro* and *in vivo* studies, which is a future step of this study. This work was carried out by accessing resources of the Computational Chemistry and Biology Network (CCBN) at the North Dakota State University.

Supported by NIH Grant P20RR016741 from the NCRR.

ANGIOGENIC GROWTH FACTORS EXPRESSION AND THE CULTURE AND CHARACTERIZATION OF CAPILLARY ENDOTHELIAL CELLS FROM EQUINE EXUBERANT GRANULATION TISSUE

Nicole Rowley* and Lynn Burgess
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Exuberant granulation is the formation of excessive amounts of granulation tissue and is commonly called proud flesh. Exuberant granulation tissue, like granulation tissue in a healing wound of second intention, consists of new-formed blood vessels and proliferating fibroblasts. The neovascularization comes from proliferating endothelial cells (EC) in a process called angiogenesis. Angiogenesis has not been well addressed in equine medicine, but is a very important factor in exuberant granulation tissue formation and is a possible contributor to the pathology of the condition. The objective of this study was to culture and characterize capillary endothelial cells (EC) from equine exuberant granulation tissue and determine the expression of angiogenic growth factor mRNAs to understand the cause of this disorder.

Exuberant granulation tissue was harvested from the back leg of a five-year-old Quarter Horse mare and the front leg of a yearling Paint colt. EC were isolated from the tissue by mincing and trypsinizing, and then characterized by the expression of von Willebrand factor and morphology. Cells were grown for over 55 passages and tested for normal growth and life expectancy by population doubling in under various culture conditions. Angiogenic growth factors were detected by separating RNA from the tissue and cultured EC, and then growth factor specific primers were used with reverse transcriptase-polymerase chain reaction (RT-PCR) to detect mRNA expression. Capillary EC were easily cultured and characterized by in vitro morphology and the expression of von Willebrand factor. The exuberant granulation tissue and EC both expressed three isoforms of vascular endothelial growth factor (VEGF); these were the amino acid isoforms 121, 165 and 189. Tissue samples expressed the secreted 121 isoform the most and the EC expressed the membrane bound 189 isoform the most. Midkine and fibroblast growth factor basic were expressed weakly in just the tissue. There was no expression of other possible angiogenic growth factors. These factors were fibroblast growth factor alpha, somastatin, platelet factor-4, thrombospondin, leukemia inhibitory factor and interferon gramma.

Exuberant granulation tissue provides an easily cultured source of capillary EC, which should be valuable in studies of the disease. The expression of VEGF by the EC shows a self-simulation that may be the pathology for this disease. VEGF was the most important angiogenic growth factor expressed and could be a possible target for treatment.

THE PRESENCE OF ATRAZINE IN RIVERS AND STREAMS IN SOUTHEAST NORTH DAKOTA

Ryan Lorenz* and Andre DeLorme
Department of Science, Valley City State University, Valley City, ND 58072

Atrazine is a herbicide used mainly in corn and soybeans. It is one of the most widely used herbicides in the world. Atrazine inhibits plant growth by interfering with the normal function of photosynthesis, thus making it an effective herbicide.

Over the last ten years atrazine has been found to have endocrine disrupting effects in a variety of animals. Endocrine disrupters are chemicals that can cause adverse affects by interfering with the body's hormones and chemical messenger system. They can affect cell growth, development, metabolism, and reproductive functions. This has lead to a growing concern over the presence of atrazine in drinking water. The U.S. Environmental Protection Agency (EPA) has placed a limit on atrazine found in drinking water at 3 parts per billion (ppb).

The only major testing that has been done for atrazine in North Dakota streams was by the U.S. Geological Survey from 1992-1995. According to the U.S. Geological Survey atrazine was present (at levels of 0.001 pbb or higher) in 90 percent of all sites sampled with concentrations found as high as 4.5 ppb. The testing was done 214 times during a three year period with concentrations found year round (1).

We hypothesize that some local species of aquatic microinvertebrate may exhibit atrazine induced effects. As part of our investigation we are documenting atrazine levels in the Sheyenne River and Wild Rice River water sheds. We test atrazine levels at four locations on each the Sheyenne River and Wild Rice River. These rivers flow through areas with the highest corn concentration in the state therefore atrazine is likely to be at higher levels. The testing is done weekly in the summer months and monthly in the winter. We use two methods for collecting samples including a depth width intergraded method and a grab method. We tested the samples for atrazine using an ELISA technique. This technique is quick and relatively inexpensive. However, its limit of detection is 0.5 ppb as compared to the 0.001 ppb for the method used by the USGS.

We determined the atrazine concentrations in 173 samples. The highest atrazine concentrations were recorded in June and July. The highest recorded atrazine concentration was the Wild Rice River on July 1, 2005 at 3 ppb. Overall we had 17 samples that had a concentration of 1 ppb or higher. The atrazine levels in the fall and winter have been below detectable levels of our technique (less than 0.5 ppb). Unfortunately, due to problems with equipment and high water conditions, we were unable to collect data for a portion of the summer. We will continue our sampling regiment through the next year in order to get a complete, continuous profile of the atrazine levels in these rivers.

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1) Tomes LH, Brigham ME, and Lorenz DL Nutrients, Suspended Sediment, and Pesticides in Streams in the Red River of the North Basin, Minnesota, North Dakota, and South Dakota, 1993-95, Mounds View, Minnesota, pp. 46, 1997.

CRANIAL DEVELOPMENT IN A MINIATURIZED FORM OF THE SALAMANDER *DESMOGNATHUS QUADRAMACULATUS*: ROLE OF TEMPERATURE AND THYROID HORMONE

Kiswendsida Claude Ouedraogo and Christopher K. Beachy
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The thyroid hormones T_3 and T_4 initiate metamorphosis in larval amphibians. Lower temperature retards larval development with consequence that metamorphosis is delayed. In assessing the roles of temperature and TH in producing geographic variation in metamorphic, Hickerson et al (1) noted that lower temperature resulted in delayed metamorphosis while immersion in 4.8×10^{-9} M TH failed to cause premature metamorphosis. However, their assessment was limited to the external morphology of the head; it remains tenable that underlying cranial morphogenesis was induced by TH exposure (2).

In order to test the hypothesis that temperature and thyroid hormone (TH) interact to affect metamorphic changes in the plethodontid salamander, *Desmognathus quadramaculatus* we grew first and second year larvae (*i.e.*, premetamorphic) in a two-factor experiment wherein temperature and TH were treatment factor. We grew larvae in temperature regimes (11 and 15) and two TH concentrations (control and 1.2 nM). Temperature treatment was via two refrigerators, and included a simulated winter (lowered 7 and 11 during winter months, and raised to original levels in Spring). Animals were scored first examined externally for metamorphosis based on gill reduction and labial fold absorption, and second doubly cleared and stained for observation of cartilaginous (alcian blue) and bony elements (alizarin red) determine if external morphology was reflected in cranial features.

There was a significant correlation between external metamorphic scoring assay and cranial metamorphosis (Fig. 1). In general low temperate retards metamorphosis, and no TH effect was observed. For the cranial features that we examined (vomer, parasphenoid, maxilla, pterygoid and the hyobranchial apparatus), high temperature caused salamanders to lose their vomerine teeth and develop vomerine bars; low temperature salamanders did not undergo this transformation. The maxilla develops at high temperature but not at low temperature. The pterygoid disappears at high temperature, and the hyobranchial changes from larval to adult morphology. There were no significant changes in cranial morphology induced by TH. This suggests that plethodontid larvae are insensitive to TH-induction of metamorphosis at early larval life history. Based on our growth data, we suggest that the use of the feeding system (*i.e.*, the gape and suck apparatus) plays a role in stimulating cranial metamorphosis.

Supported by NIH Grant # P20 RR016741 (NCRR — INBRE Program)

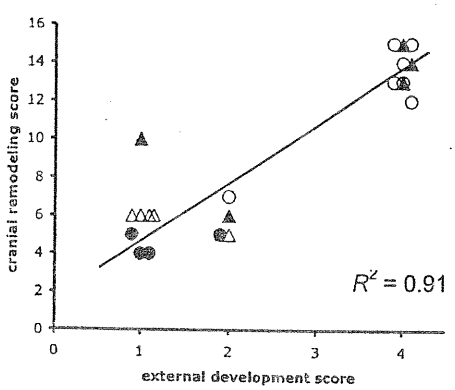

Fig. 1. – Cranial modeling in *Desmognathus quadramaculatus* plotted as a function of external metamorphic changes drawn from Hickerson *et al.* (1). Open circles are salamanders raised at high temperature that did not receive TH; closed circles -- low temperature, no TH; open triangles -- low temperature and TH; closed triangles -- low temperature and TH.

Sources:
1) Hickerson CM, Barker EL, Beachy CK. *Southeastern Naturalist* 4:33-50, 2005.
2) Rose CS. *J. Morphology* 223:149-166, 1995.

THE EFFECTS OF LYCOPENE ON THE CELL PROLIFERATION AND EXPRESSION OF CONNEXIN PROTEINS mRNAs OF BOTH NORMAL AND CANCEROUS CELLS

Josh Seekins* and Lynn Burgess
INBRE Program, Department of Natural Sciences, Dickinson State University, Dickinson, ND 58601

Strong epidemiological data indicate that lycopene may have anticarcinogenic properties. Several studies have demonstrated that serum levels of lycopene and consumption of its dietary sources were inversely related to the incidence of cancer, with the strongest evidence being seen with prostate cancer and some affect with cancers of the bladder, pancreas, stomach, and lungs. Lycopene has been promoted in the media and sold as a dietary supplement with claims of anticancer and other health benefits. Presently, the mechanism of lycopene action has not been established. The efficiency and safety of pills containing lycopene have not been evaluated.

Gap junction communication (GJC) is the communication between adjacent cells through small pores. Small molecules (less than 1.5 kDa) and ions are also able to pass from cell to cell through these pores. Gap junctions are composed of two symmetrical structures-one from each cell-called connexons. Connexons are composed of gap junctional proteins called connexins (cx). The cx family includes approximately 17 proteins which are named based upon their molecular weight. It is believed that lycopene's anticarcinogenic properties are due to its ability to enhance or renew gap junctional cx proteins. GTC is one of the very last functions lost during the transformation and development of a cancer. Normal cells are capable of suppressing an adjacent cancer cell from proliferating.

A long-term goal of this study is to investigate the anticarcinogenic properties of lycopene at a cellular level. Our hypothesis is that one of the lycopene's anticarcinogenic properties is due to its ability to suppress the proliferation of cancer cells and to determine if lycopene treatment of cultured human cells will affect the expression of the mRNAs for the cx proteins.

Following cell lines: DU-145, human prostate adenocarcinoma cells; HS-68, human foreskin fibroblast cells; A549, human lung carcinoma cells; HS-578T, human breast carcinoma cells; RWPE-1, human prostate cells; IMR-90, human lung cells were seeded lightly on 24-well plates and incubated for 24 hours, then the media was removed and media with lycopene and 1% acetone was added at a dose of 10^{-10} M to 10^{-5} M. Cells were incubated for 24, 48, and 72 hours, and then counted electronically and compared to untreated controls.

None of the cell lines showed any significant changes to their proliferation at any of their sample times contrary to other published reports. All the cells grew normally and continued to proliferate through the 72 hours of incubation.

Cell lines were grown in culture to 100% confluency, and then treated with lycopene with a dose range from 10^{-10} M to 10^{-5} M for 48 hours. RNAs were extracted and converted to cDNAs. With the use of standard PCR, 17 connexins mRNAs were tested for their expression in the cell lines.

Generally there was a strong expression of mRNAs in cx 26, 30, 31, 32, 36, 37, 40, 43, 45, 50, of most cell lines and a weak expression of 30, 31, 32, 36, 37, 50, and 59 in some cells and no expressions from 46 and 47 in any cell lines.

DOES SIZE REALLY MATTER? THE EFFECTS OF ATRAZINE AND COMPETITION ON GROWTH IN THE SALAMANDER (THE AXOLOTL, *Ambystoma mexicanum*)

Francis Drew Henry* and Christopher K. Beachy
Department of Biology & Amphibian Growth Project, Minot State University, Minot, ND 58707, USA

We used the ambystomatid salamander *Ambystoma mexicanum* (the axolotl) to test the hypothesis that atrazine, in combination with density as an environmental stressor, affects growth, development and gene expression. Axolotl makes an excellent toxicological model system: they have high fecundity, have minimal housing requirements relative to other model vertebrates, include genomic and bioinformatics resources (1), and are part of a group of lineages that are broadly distributed among North American habitats that are exposed to endocrine disrupting chemicals (2).

We used density as a natural environmental stressor in combination with sub-lethal doses of atrazine. Hatchling axolotls were fed brine shrimp, each box receiving an equal volume (eg. 1 aliquot), with numbers of aliquots increasing as axolotls from single density treatments grew. When the axolotls were big enough to consume black worms as food, we placed 3.5 grams (wet mass) in each box. We assayed growth by weighing the axolotls at four time points. We analyzed growth data that were collected periodically throughout the 3 months of the experiment. Our experimental design consisted of 195 salamanders that were exposed to 9 treatments of varying densities and atrazine concentrations in 5 separate blocks, all contained within a single cooler to regulate environmental conditions. Atrazine treatments include a control, 1 ppb, and 5 ppb; densities were 1, 4 or 8 salamanders per box.

Increased density caused a significant decrease in growth. No effects of atrazine or its interaction with density on growth were detected. Gene expression profiles were significantly different among density treatments, but no impact on gene expression resulted from atrazine treatments. Inspection of gonadal development was not completed at this report.

Density of conspecifics (and the consequent competition) has predictable and direct effects on growth in natural communities of ambystomatid salamanders (3). Previous results have demonstrated a lowered metamorphic size in ambystomatids treated with atrazine. While atrazine causes endocrine-disruption (e.g., gonadal deformities) in amphibians, our evidence suggests that it may not have effects on non-endocrine systems, such as larval growth.

This research was supported by NIH Grant Number P20 RR016741 from the INBRE Program of the National Center of Research Resources.

Sources:

1) Putta S, *et al. BMC Genomics* 5:54, 2004.
2) Lannoo M. (ed.). *Amphibian Declines: The Conservation Status of United States Species*. Berkeley, CA: University of California Press. 1094 pp., 2005.
3) Scott DE *Ecology*, 71:296-306, 1990.

PHENOTYPIC CHARACTERIZATION OF A *YERSINIA ENTEROCOLITICA FLHD* MUTANT

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Background: *Yersinia enterocolitica* is an enteric bacterium that is closely related to *E. coli* and has potential for pathogenicity. FlhD/FlhC is a global regulator of metabolism (1). Gene array technology can detect the control FlhD/FlhC has on the expression levels of genes, but cannot predict the phenotypic outcome. Here we report the use of phenotype microarray technology to detect the phenotypes of a *flhD* mutant grown on various carbon and nitrogen sources and at different pH values.

Methods: Various compounds relevant to carbon and nitrogen metabolism were contained individually in the wells of 96-well microtiter plates of the Phenotype MicroArray system (Biolog, Hayward, CA). Plates used for the pH experiments had wells containing various pH values. Suspensions of wild-type *Y. enterocolitica* and its isogenic *flhD* mutant were produced from cells grown on minimal medium R2A plates, administered to each well (2), and incubated at 4°C, 25°C or 37°C. Growth ratios between the wild-type and *flhD* mutant were calculated by dividing the average growth of the wild-type by that of the *flhD* mutant. Pathways used to degrade nutrient sources that provided differential growth conditions for the two strains were reconstructed with the Kyoto Encyclopedia of Genes and Genomes (KEGG: www.genome.jp/KEGG). To confirm our data, we performed quantitative PCR with a selection of genes that were involved in these pathways.

Results: For the carbon sources, the wild-type cells exhibited better growth with intermediates of the upper glycolytic pathway (N-acetyl-D-glucosamine, and D-mannose). The *flhD* mutant grew better with intermediates of the lower glycolytic pathway (acetic acid and D-alanine), compounds metabolized by the TCA cycle (L-asparagine), and purines (adenosine, deoxyadenosine, and inosine). On nitrogen plates, several pyrimidines (cytosine, cytidine, thymidine, and uracil) provided better growth conditions for wild-type cells at 25°C. The only other nitrogen source that provided better growth conditions for the wild-type cells was L-histidine. The *flhD* mutant grew better on nitrogen sources that feed into the urea cycle (L-arginine, L-proline, D-alanine, and ammonia) at 4°C.

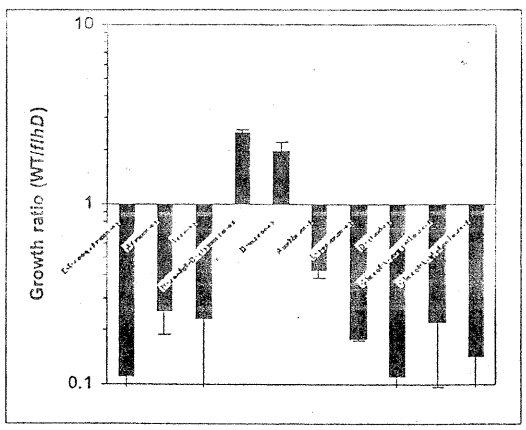

Fig. 1: Growth on carbon sources at

In addition, several dipeptides provided differential growth conditions for the two strains. Little difference in growth was observed between the two strains under different pH conditions. Both strains grew up to pH 10 at 25°C. Quantitative PCR confirmed the regulation by FlhD/FlhC of ten genes that were involved in pathways that were involved in nutrient sources that provided differential growth conditions for the two strains.

Conclusion: Phenotypes correlate with the previously observed gene regulation. FlhD/FlhC seems to balance the flux of nitrogen in a way that pyrimidine metabolism is enhanced and purine metabolism is repressed. In addition, FlhD/FlhC might enhance the upper half of glycolysis and repress the bottom half of glycolysis, the TCA cycle, and the urea cycle.

Kapatral V, Campbell JW, Minnich SA, Thomson NR, Matsumura P, Prüß BM. *Microbiol.*, 150, 2289, 2004.
Bochner B, Gadzinski P, Panomitros E. *Genome Res.*, 11, 1246, 2004.

CLASSIFICATION OF EMBRYO SIZE IN THE FEDERALLY LISTED THREATENED PLANT, THE WESTERN PRAIRIE FRINGED ORCHID (*PLATANTHERA PRAECLARA*)

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The western prairie fringed orchid (*Platanthera praeclara* Sheviak and Bowles) has been known to inhabit the Sheyenne National Grasslands in southeastern North Dakota for years. It is North Dakota's only federally listed threatened plant. The preservation of this remnant population is a high priority. Seed production and seed viability estimates are a critical part of survival modeling for this species.

This study was designed to develop a system to classify viable embryos. The current system classifies viable embryos based on their description as ovoid and hyaline. This has caused confusion among researchers as to what to consider viable. Small embryos (no numerical measurement known) are considered not capable of germinating by some researchers. According to From (1) a large embryo is hyaline, round or ovoid and consumes over half the space within the testa and is assumed to be capable of germinating. Small embryos are hyaline, round or ovoid and consume close to half the area within the testa and may or may not be capable of germination but are included in her estimates of viable seeds.

Using the 40 power objective on a compound light microscope, the length and width of the testa (structure surrounding the seed) and the length and width of 100 ovoid hyaline embryos were measured. It was determined that an average large embryo measures 1.9 by 1.1 μ and a small embryo measures 1.5 by 0.9 μ (Table 1). We hypothesize that any structure within the testa less than 0.9 by 0.5 μ is most likely not capable of germinating because all structures less than that size in our study were neither ovoid or hyaline. We hypothesize that ovoid hyaline embryos larger than 0.9 by 0.5 μ can be assumed to be capable of germinating, given the proper environmental conditions. The next step in this research is to test this hypothesis using a chemical viability analysis (which destroys the embryos) to compare the viability of embryos of varying sizes.

Table 1. Analysis of measurements of ovoid, hyaline western prairie fringed orchid embryos assumed to be viable.

	Small embryo	Large embryo
Mean length & width (μ)	1.5 x 0.9	1.9 x 1.1
SD length & width (μ)	0.3 x 0.2	0.2 x 0.2
Range for length	0.9 - 2.0	1.2 - 2.7
Range for width	0.5 - 1.2	0.9 - 1.7

[1]**Margaret From, Senior Scientist, Henry Doorly Zoo, Omaha, NE (personal communication)**

DOES DNA TOPOISOMERASE II BIND IN THE *MLL* TRANSLOCATION BREAKPOINT CLUSTER REGION?

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Leukemia cells, and those of other cancers show many types of chromosome abnormalities, such as missing or extra chromosomes and chromosome translocations. The *MLL* gene is important because of the frequent involvement of this gene in chromosome translocations in acute leukemia, especially in children less than one year of age . *MLL* rearrangements are observed in both primary leukemias as well as secondary leukemias, often in cases in which the patient has been treated for a primary tumor with a DNA Topoisomerase II (Topo II) inhibiting drug (1). All *MLL* translocations show a conserved breakpoint cluster region (bcr) of 8kb near the 5' end of the *MLL* gene.

Topo II is a nuclear protein that is essential for DNA replication and transcription. The enzyme acts to relieve torsional stress in DNA by creating a double-strand break, allowing passage of a second region of double-stranded DNA to pass through and resealing the break. Topo II inhibitors used in cancer treatment inhibit the sealing of the double strand breaks. Cells exposed to Topo II inhibitors accumulate DNA double strand breaks which ultimately induces apoptosis. The correlation of Topo II inhibition and *MLL* translocations in secondary leukemia suggests a possible role for this protein in binding and breaking DNA within the 8 kb translocation breakpoint. Several in vitro studies have shown the ability to induce breaks in *MLL* using common Topo II inhibitors. (2). However no one has shown that Topo II binds directly within the *MLL* bcr.

In this study, we have used a chromatin immunoprecipitation (ChIP) assay to determine if Topo II binds in the *MLL* bcr. ChIP is an assay which allows one to determine the in vivo binding sites for DNA binding proteins by cross-linking the bound proteins to chromatin in intact cells. In our assay, we have treated human leukemia cell lines with normal *MLL* genes (intact *MLL* bcr) with formaldehyde to cross-link and stabilize all protein-DNA interactions. Cells are lysed and DNA is sheared into small, uniform fragments. Sheared chromatin is then immunoprecipitated with Topo II or control antibodies and assayed by polymerase chain reaction (pcr) to determine whether Topo II bound in the *MLL* bcr.

In the course of this study, we have optimized shearing conditions using two methods as well as conditions for PCR analysis using two different controls. Preliminary results suggest that Topo II may bind in two regions, one at the extreme 5' end of the bcr and one that is near a proposed Topo II cleavage site, but not in other regions of the BCR. However, detection of Topo II binding in these regions is not as prominent or as consistent as in the control assay.

Initial studies were performed in the absence of any TopoII-inhibiting drugs. In the current phase of study, we have begun the same analysis, but have treated our cells with etoposide, one of the most commonly used TopoII targeting chemotherapy drug used in cancer treatment . Our goal is to confirm binding or recognize differences in TopoII affinity to the *MLL* BCR in the presence and absence of Topo II inhibitors. Preliminary results will be discussed.

1)Gill Super HJ, McCabe NR, Thirman MJ, Larson RA, LeBeau MM, Pederson-Bjergaard J, Preben P, Diaz MO, Rowley JD *Blood* 82:3705-3711, 1993.
2) Aplan PD, Chervinsky DS, Stanulla M, Burhans C *Blood* ,87: 2649-2658, 1996

Supported by NIH grant P20 RR016741 from the NCRR.

EFFECT OF CHLOROPHENOXY HERBICIDES ON THE TRACHEAL DEVELOPMENT OF *DROSOPHILA MELANOGASTER* EMBRYOS

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Recent publications have linked exposure to several herbicides in areas of high wheat production with an increase in the occurrences of respiratory birth defects. One of the herbicides used routinely in the maintenance of wheat is 2,4-Dichlorophenoxyacetic acid (2,4-D). 2,4-D has not been found to be severely toxic in classical toxicity experiments, but recently has been classified as an endocrine disruptor. Endocrine disruptors can affect hormonal pathways and lead to abnormal growth, even when exposed at low non-toxic levels. *Drosophila Melanogaster* embryos were used as a model, because of the similarities between the Fibroblast Growth Factor (FGF) pathways involved in tracheal development in the *Drosophila* and lung development in humans. We hypothesize that exposure to 2,4-D during early embryonic development disrupts the FGF pathway which may lead to disruptions in tracheal development.

Drosophila embryos were collected for twenty minutes after an hour pre-lay and exposed to heptane for 20 seconds and then exposed to the 2,4-D (3 mM) for five minutes. After 12 hours a tracheal staining protocol was used to visualize the tracheal development in each embryo by using the 2A12 primary antibody, secondary antibody, ABC system, and DAB. As a negative control for the staining, the staining protocol without the primary antibody was used. Subsequently, tracheal development of each embryo was categorized as either normal or disrupted using a Nikon inverted microscope. The slides were evaluated by three individuals (blindly, to prevent bias) to find the average of tracheal disruptions. The experiment was repeated four times.

The average number of disrupted tracheal systems in normal embryos was 33.5%± 14.7 whereas the exposed embryos had a disruption of 51.3% ± 14.0 ($p<0.06$). This was on average a 1.7 fold increase in disruption compared to non exposed embryos (ranging from 1.1-3.0). These results show that 2,4-D affects the tracheal development of *Drosophila Melanogaster* embryos, which supports our hypothesis that exposure to 2,4-D disrupts tracheal development. More experiments are needed to explore the mechanism.

Supported by NIH Grant P20RR016741 from the NCRR.

IDENTIFICATION OF UNKNOWN MARINE CYANOBACTERIA FROM THE GENUS *SPIRULINA*

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Cyanobacteria are prokaryotes that are capable of oxygenic photosynthesis. Sometimes referred to as blue-green algae, cyanobacteria are one of the few bacteria capable of nitrogen fixation. Some species form microbial mats that are detrimental to marine aquaria. Little is known on the species composition or the environmental conditions that facilitate the formation of these mats. We have identified a new species of *Spirulina* as a major component of these mats. We are currently attempting to bring this species into pure culture and determine its optimal growth conditions.

Sample Collection. *Spirulina* samples were collected from a marine aquarium and grown in filter-sterilized seawater, 29.4 mM $NaNO_3$, 8 mM urea, 500 µl vitamins and 100 µl trace elements. Flasks received 24 hrs. of 25.5 µE of white light from broad-spectrum fluorescent bulbs and were grown at 28°C at 100 rpm. *Spirulina* were transferred via Pasteur pipette mounted in micromanipulator in attempt to transfer only a few filaments to reach a pure culture.

Nucleic Acid Extraction, PCR, Cloning, and Sequencing. Nucleic acids were extracted as previously described (1). Fragments of 16S rDNA from *Spirulina* were PCR amplified by using cyanobacteria specific primers cyo106F to cyo781R under standard conditions and cloned into pCR 4.0 vectors per manufacturer's directions (Invitrogen, Carlsbad, CA). Phylogenetic analysis was done using a neighbor-joining algorithm.

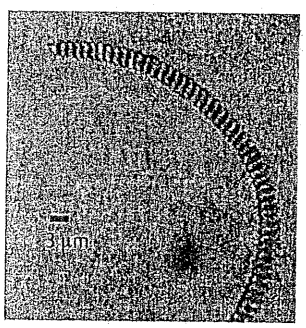

Figure 1. Light micrograph of *Spirulina* sp.

We are currently cultivating a previously unknown species of *Spirulina* at a 3.1% salinity. This particular species was capable of growing in salinities from 3.1% to 3.6%. *Spirulina* are also able to grow in fresh and marine environments with salinities from 0% to 16%. This new species of *Spirulina* are very tolerant of high urea concentrations, 8 mM, whereas the most closely related species in pure culture, *Arthrospira platensis*, has been shown to only tolerate a urea concentration of 1.7 mM (2). This particular species of *Spirulina* is very red in color indicating the possible presence of phycoerythin, found only in three species of *Spirulina*, as the main light harvesting pigment. It forms a tightly coiled right-handed helix with a cell width of 1.5 µm and trichome width of 3 µm (Fig. 1). The trichome is motile by means of cryptic motility, which causes the trichome to rotate in a counter clock-wise direction.

We amplified and cloned a 683 base pair fragment from this *Spirulina* species using cyanobacteria specific primers. Phylogenetic analysis revealed that this *Spirulina* species was found to have a 98% identity with species MPI S4 and a 97% identity with species P7 (Fig. 2).

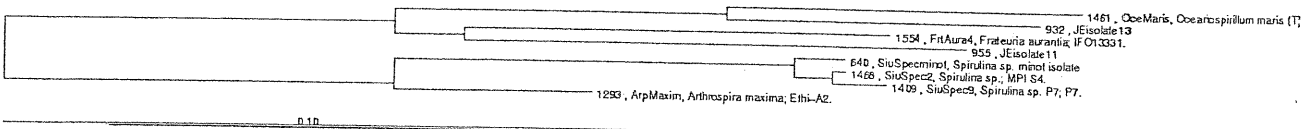

Figure 2. Phylogenetic associations among *Spirulina* species. Bar represents nucleotide substitutions per position.

Each of these species are phenotypically similar, being right-handed helices with very similar trichome and helix width measurements. Species MPI S4 and P7, however, appear blue-green when observed in white light and are reported to be incapable of chromatic adaptation (3).

References

1) Lepp PW, Brinig MM, *et al* *Proc Natl Acad Sci USA*, 101(16), 6176-81, 2004.
2) Converti A, Scapazzoni S, Lodi A, Carvalho JC *J Ind Microbiol Biotechnol*, 33(1), 8-16, 2006.
3) Nübel U, Garcia-Pichel F, Muyzer G *Int J Syst Evol Microbiol*, 50, 1265-1277, 2000.

LEAF ATTACHMENT APPEARS REQUIRED FOR INHIBITION OF LEAF EXPANSION IN ARABIDOPSIS BY THE PLANT HORMONE INDOLE-3-ACETIC ACID

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Indole-3-acetic acid (IAA) is a hormone responsible for controlling aspects of plant growth. In stems it is involved in vascular development and leaf initiation where the hormone promotes growth. Previous work has shown that IAA may also play a role in leaf expansion. Increasing the IAA content of intact expanding leaves of *Arabidopsis* and *Phaseolus*, either through exogenous application or through trapping the endogenous hormone in leaves, results in inhibition of leaf growth.[1] Paridoxicaly, other work has clearly shown that treatment of excised leaf strips from tobacco (*Nicotiana*) with IAA stimulates rather than inhibits growth.[2] IAA treatment, whether of intact or of excised leaf tissue results in epinastic (downward) curvature due to relatively greater growth by the adaxial (dorsal) side of the tissue.

The current, not yet complete, project attempts to determine the growth and epinastic sensitivity of attached leaves, excised leaves, and of excised leaf strips of the model plant system *Arabidopsis*. We also are attempting to determine whether the reversed sensitivity to IAA seen in earlier leaf strip versus intact plant experiments is the result of a wound response resulting from excision or to detachment from the plant.

For our experiments, stratified *Arabidopsis* seeds were sown in 288 well plug trays containing moist potting soil (Schultz Co., Bridgeton, MO) and grown in a growth chamber at 19°C, with continuous illumination (150 $\mu M\ s^{-1}\ m^{-2}$). After 10-14 days, plants were selected with both the first two true leaves 2.7-3.3 mm in diameter. For one set of experiments, scaled digital images of the intact attached leaves were prepared for subsequent determination of initial leaf area. For another set of experiments detached leaves were imaged. Initial images of excised leaf strips (0.7 mm wide cut transversely across the midpoint of the leaves) were prepared for a third set of experiments and for a fourth set of experiments wounded attached leaves (sliced transversely from leaf edge to near the midvein in three places) were imaged. For all experiments one of the first two leaves (selected randomly) from each plant served as the experimental (IAA treated) leaf and the other leaf served as a paired control. Treatment solutions included: full strength Murashige and Skoog media (Caisson Laboratories, Rexburg, ID), 10 mM KCl, 0.1 mM Mes/Btp (pH 6.0), +/- IAA at various concentrations (10 mM, 50 mM, 100 mM, 300 mM, and 1 mM). Following initial imaging attached leaves and wounded attached leaves received either a 5ml drop of a control treatment solution (minus IAA) or a 5 ml drop of the treatment solution containing IAA. Detached leaves and leaf strips were each incubated on 3 mL of the same solutions. One day (24 hours) later, the attached leaves and wounded leaves were detached from the plant and again imaged as were the detached leaves and excised leaves. Epinatic curvature of leaves was measured as the difference between area of the leaf as imaged lying on a glass slide and the area of the same leaf flattened by the weight of a 4.7g glass slide. Estimation of epinastic strip curvature was estimated from profile images as previously described. [1]

The growth of intact attached leaves was found to be somewhat insensitive to IAA. While lower concentrations of IAA were ineffective, 300 mM and 1 mM were inhibitory. For example, at 300 µM the area of IAA treated leaves increased 51.7 +/- 10.1% compared to 77.2 +/- 6.3 % for the controls (n=12).
IAA induced epinasty was also at the higher concentrations. At 300 mM, IAA-treated leaves showed 36.0 +/- 11.9% curvature area (mm^2) while control leaves had only 6.7 +/- 6.0% area (mm^2). Leaves treated with 1mM IAA had 51.5 +/- 2.9% curvature area as compared to 10.3 +/- 3.1% in the controls (n=7).

The growth of detached leaves floated on IAA was sensitive to the same high concentrations but here growth was increased. Significant epinasty was also induced. As expected leaf strips incubated in IAA grew significantly more than control leaves across a range of concentrations 10 mM and higher. Significant leaf strip epinasty was induced by IAA at the same concentrations. Wounded attached leaves were significantly inhibited by IAA applied at 50 mM, the only concentration so far tested.

The data so far collected suggests that leaf attachment is required for IAA induced growth inhibition. Epinastic curvature, seen in both growth inhibition and growth induction, must result for different growth sensitivities of cell layers across the width of the leaf.

This project is supported by NIH grant P20 RR016741 from the NCRR

[1] Keller CP, Stahlberg R, Barkawi L, Cohen JD (2004) Plant Physiology 134: 1217-1226, 2004.
[2] Keller CP, Van Volkenburgh E (1997) Plant Physiology 113: 603-610, 1997.

A LOWER PRESSURE PROCEDURE FOR MICROWAVE ASSISTED SYNTHESIS OF NOVEL FORMAMIDE FUNGICIDES

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Turtle Mountain Community College, Belcourt, ND and [§]Minot State University, Minot, ND

Formamides are a novel group of medicinal and agricultural fungicides. Some of these novel fungicides were obtained by reductive amination via Leuckart reaction, where formamide was used both as a reagent and a solvent. During the early development stage it was shown that the reaction requires up to 1 hour at the temperature range of 180-190°C to be completed. Later it was found that microwave assistance allows the reaction to be completed in three minutes at 200°C. However, a high pressure in the range of 150 - 200 PSI develops in the microwave assisted reaction which requires the use of expensive reaction apparatuses for microwave assisted syntheses. In this work the use of water as an additive to formamide was investigated, and it was discovered that in the presence of water, the microwave assisted reaction produces significantly lower pressure, within the range of 50-70 PSI. The lower pressure procedure for microwave assisted Leuckart reaction provides a safer way for the synthesis of novel formamide fungicides and allows the use of less expensive equipment.

ESTABLISHING INHIBITORY CONCENTRATIONS OF NOVEL FORMAMIDE FUNGICIDES ON FUNGI PERTINENT TO MEDICINE AND AGRICULTURE

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Formamides are a novel group of anti-fungal drugs discovered by Bobylev *et al.* Formamides were designed as analogs of triazole fungicides where the triazolyl moiety is replaced with the formamide moiety. Being analogs, formamide fungicides are similar to triazole fungicides in both the spectrum and the level of anti-fungal action. However, it was shown later that formamide fungicides differ form triazole fungicides in their biochemical mode of action. Specifically, it was found that the lead candidate N-[1-(2,4-dichlorophenyl)-4,4-dimethylpent-1-en-3-yl] formamide (I) did not affect the sterol composition of fungi that were grown in the presence of inhibitory concentrations of I. The site and mechanism of the anti-fungal action of novel formamide fungicides remains unknown. One of the ways to determine the biochemical mode of action of a new fungicide is to investigate the changes in secondary metabolite production by the affected organisms. In this work, inhibitory concentrations were established against a number of medicinal and agricultural fungal pathogens. These concentrations will be used in future projects to determine the changes in the secondary metabolic profiles of the affected fungi and ultimately to determine the biochemical mode of action of novel formamide fungicides. Development of fungicides that target different modes of action is vital to overcoming anti-fungal resistance.

This project was supported by NIH grant P20 RR016741 from the NCRR.

ALPHA-2A ADRENERGIC RECEPTOR-MEDIATED INHIBITION OF RAT HIPPOCAMPAL EPILEPTIFORM ACTIVITY

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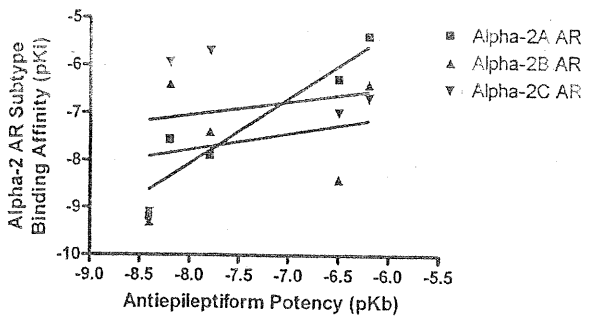
The endogenous neurotransmitter norepinephrine (NE), has been implicated in neural functions as diverse as sleep, learning and memory, depression and epilepsy. Data from animal studies indicate that NE has potent anticonvulsant properties. Our preliminary evidence suggests that NE has a biphasic excitatory/inhibitory effect on hippocampal CA3 network activity and is exerting its anticonvulsant actions by activating an alpha-2 adrenergic receptor (AR) expressed on the pyramidal cells of the hippocampal CA3 region (1). Pharmacological and molecular cloning studies have revealed the existence of three alpha-2 ARs: 2A, 2B and 2C (2). The genes encoding these ARs have been cloned, confirming that each receptor class comprises a family of homologous proteins with unique characteristics. We hypothesize that a *specific subtype of alpha-2 AR is mediating the inhibitory actions of NE on epileptiform burst discharge activity.* Rat hippocampal slices were prepared according to a protocol approved by our Institutional Animal Care & Use Committee. Extracellular field potentials were recorded from the stratum pyramidal of the CA3 region of the neonatal rat hippocampal slices using a glass electrode filled with NaCl. Epileptiform burst discharge activity was induced using the GABAergic blocker picrotoxin, and concentration-response curves were created by bath applying increasing concentrations of epinephrine (EPI), a substitute for NE, to the hippocampal brain slices *in vitro*. Varying doses of subtype-specific alpha-2 AR antagonists were utilized to create individual EPI concentration-response curves that were compared using Schild regression analysis (3) to determine the effects of the different concentrations of antagonists and to isolate each specific AR subtype. The pA_2 values obtained correlated best with affinity values for the alpha-2A AR (see Figure). These results suggest that it is primarily the alpha-2A AR which is mediating the inhibition of epileptiform burst discharge activity in this model. The inhibition of epileptiform activity through the activation of the adrenergic system has potential in that it has been shown to modulate seizure susceptibility without the deleterious side effects of the drugs currently used to treat epilepsy. Further insight into this mechanism will help to explain how NE modulates neural networks and may lead to possible exploitations for therapeutic use.

Funding: This study was supported in part by ND EPSCoR through NSF grant EPS-0447679 (VAD), CAREER Award 0347259 (VAD), NIH grant 5P20RR017699 from the COBRE program (JEP, VAD), University of North Dakota (UND) New Faculty Scholar Award (VAD), Epilepsy Foundation Predoctoral Research Training Fellowship (CWJ), ND EPSCoR Advanced Undergraduate Research Award and an Undergraduate Premed/Medical Student Summer Research Award (JAL) from the UND Office Associate Vice President for Health Affairs & Medical Research.



References:
1) Jurgens CW, Boese SJ, King, JD, Pyle SJ, Porter JE, Doze VA *Epilepsy Res* 66:117-128, 2005.
2) Bylund DB, Eikenburg DC, Hieble JE, Langer SZ, Lefkowitz RJ, Minneman KP, Molinoff PB, Ruffolo RR Jr, Trendelenburg U *Pharmacol Rev* 46:121-136, 1994.
3) Arunlakshana O, Schild HO *Br J Pharmacol* 14:48-58, 1959.

APPLICATION OF FLUORESCENT PROBE LIFETIMES TO IMPROVE RT-PCR AND SNP DETECTION METHODOLOGIES

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Our research group has implemented novel fluorescence measurement technology developed by our collaborators at Dakota Technologies, Inc. Wavelength-time matrices (WTM's) consisting of a series of fluorescence decay waveforms at multiple emission wavelengths are collected rapidly through the use of short pulses of light from a microchip laser and a very fast digitizer. The information contained within these WTMs is relevant to many biomedical research applications, including DNA sequencing, SNP detection, multiplex PCR, and drug-target binding.

Our current research is aimed at demonstrating that fluorescence lifetime methodology can dramatically improve traditional RT-PCR and SNP detection techniques and allow for multiplexed applications. Initial studies of identifying a series of fluorescent lifetime probes have been performed using samples with a known point mutation of the human *b*-globin gene causing sickle cell anemia. The detail methodologies and results will be presented.

This research was supported by the North Dakota INBRE grant.

MOPRHOLOGY AND VASCULARIZATION OF AUTOTRANSPLANTED OVINE OVARIES AFTER FREEZING AND THAWING

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Department of Animal and Range Sciences, North Dakota State Univ., Fargo, ND and
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Therapeutic advances in the treatment of cancer are leading to improved survival and effective cure. However, treatment of cancer with radiotherapy and chemotherapy predispose ovaries to failure or permanent damage. Therefore, procedures of ovarian tissue preservation are being developed and/or improved. In this study, sheep were used as a model to further improve and evaluate autotransplantation of frozen-thawed ovaries. The objective of this study was to evaluate the morphology, vascularization, expression of vascular endothelial growth factor (VEGF; one of the major angiogenic factors), and cellular proliferation and apoptosis in autotransplanted ovine ovaries after freezing and thawing.

Mature crossbred ewes were divided into two experimental groups; an intact (control) group (n = 5 ewes), and treatment group (n = 4) in which oophorectomy was performed laparoscopically and ovaries with intact vascular pedicles frozen. After 1 week, ovaries were thawed and transplanted back into the same animal at a different site. Four months after autotransplantation, all ewes were treated with progesterone sponges for 14 days to synchronize estrus. Estrus was detected using a vasectomized ram. All ewes were treated with follicle stimulating hormone on days 13 and 14 of the estrous cycle to induce multiple follicle development. On day 15 of the estrous cycle, ovaries were removed surgically from the transplanted site in the treated animals and from the pelvis in the controls. After separation of the cumulus oocyte complexes, ovarian tissues were fixed in Carnoy's solution and/or 10% formalin for histological and immunohistochemical evaluations. Both fully functional ovaries were collected from each control ewe. However, out of eight autotransplanted ovaries, a total of two functional ovaries were found and collected from two ewes (one ovary/ewe). Fixed ovaries were embedded in paraffin, sectioned and used for 1) evaluation of morphology by using hematoxylin and Schiff's reagent staining, 2) detection of blood vessels by immunolocalization of factor VIII (marker of endothelial cells), VEGF (expressed by pericytes and smooth muscle cells) and smooth muscle cell actin (SMCA; marker of smooth muscle cells), 3) determination of the ability of cells to proliferate by staining for the presence of proliferating cell nuclear antigen (PCNA), and 4) detection of apoptotic cells using the TUNEL method.

The morphology of control and autotransplanted ovaries was similar. In control and autotransplanted ovaries, primordial, primary, secondary (antral) and preovulatory follicles were found along with fully functional vascularization which was manifested by expression of factor VIII, VEGF and SMCA. Proliferating cells were detected in ovarian follicles, and the rate of apoptosis was minimal in both control and autotransplanted ovaries.

These data demonstrate that ovarian function was restored after autotransplantation of frozen and thawed ovaries, since follicles from several stages of folliculogenesis and blood vessels expressing specific markers of vascularization were present. In addition, ovarian cell proliferation was restored and apoptosis was minimal. Thus, autotransplantation of an intact frozen-thawed ovary is feasible and allows for restoration of vascularization and cellular function. However, additional improvements are required to enhance the efficiency of autotransplantation of frozen-thawed ovaries. *Supported by grant from the Cleveland Clinic Foundation, (RPC # 07688).*

SCHEDULE OF PRESENTATIONS — Auditorium — VCSU Science Center (Room 128)

Denison Competition – Graduate Communications – Chris Keller - Moderator

10:40 a.m. - ISOLATION, IDENTIFICATION AND CHARACTERIZATION OF *Enterobacter sakazakii* FROM BOVINE AND BISON FECAL SAMPLES IN NORTH DAKOTA. Lilian Nangoh*, Heather Vinson, Margaret Khaitsa and Penelope Gibbs, Department of Veterinary and Microbiological Science, Great Plain Institute of Food Safety, North Dakota State University

11:00 a.m. - FIBRONECTIN FRAGMENTS EXPOSE THE EVIL SIDE OF INTEGRIN SIGNALING AND ENHANCE OSTEOARTHRITIC DAMAGE. Lei Ding*, Guo Danping, and Gene Homandberg, *Dept. of Biochemistry and Molecular Biology, School of Medicine, University of North Dakota, Grand Forks*

11:20 a.m. - HATs OFF TO TRANSCRIPTION. Lata Balakrishnan* and Barry Milavetz, *Department of Biochemistry and Molecular Biology, University of North Dakota*

11:40 a.m. - UPREGULATION OF TRPC1 FOLLOWING SERCA2 GENE SILENCING PROMOTES CELL SURVIVAL: A POTENTIAL ROLE FOR TRPC1 IN DARIER'S DISEASE. Biswaranjan Pani*, Eric Cornatzer, Brij B Singh, *Department of Biochemistry and Molecular Biology, School of Medicine & Health Sciences, University of North Dakota*

ISOLATION, IDENTIFICATION AND CHARACTERIZATION OF *Enterobacter sakazakii* FROM BOVINE AND BISON FECAL SAMPLES IN NORTH DAKOTA

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Enterobacter sakazakii continues to be implicated in neonatal infections such as meningitis and necrotizing enterocolitis. Powdered infant formula has been reported to be the most common source of contamination for infected neonates (1). The natural habitat of this opportunistic pathogen and its virulence traits are currently not well understood. In this study, 142 fecal samples from normal cows, cattle and bison were examined for the presence of *E. sakazakii* using a variety of techniques. The objectives of this study include: 1) to determine if *E. sakazakii* is present in bovine and bison fecal samples; 2) To determine if the recovered isolates as well as those from infected neonates and from other sources possess some putative virulence factors associated with the neonatal infections mentioned above; and 3) To assess the pathogenic potential of these isolates using the ELA.

Methods. Fecal samples used in this study were obtained from normal cattle and bison as submitted to the NDSU Veterinary Diagnostic Laboratory for Johne's disease testing. One gram of fecal matter was diluted in 10 mL PBS, pH 7.4. This was homogenized and 0.1 mL was plated on DFI agar (Oxoid Ltd., England) or on ESPM (R& F Products, IL) (1,2). All plates were incubated at 37°C overnight (O/N). The limit of detection for DFI and ESPM agars was determined. Suspect bacteria were identified using the Sensititre® GNID identification plate for gram-negative organisms (TREK Diagnostics Inc., Westlake, OH). Polymerase chain reaction was accomplished using the 16S rRNA gene sequence of *E. sakazakii* (3). In addition, the presumptively positive isolates and known *E. sakazakii* strains from the American Type Culture Collection (ATCC) were tested for multiple putative virulence factors. The ELA was performed essentially as described by Gibbs et al., 2003 except that the embryos were inoculated at 16-days incubation. In each of the three trials, PBS-inoculated, uninoculated, A4 (an avirulent negative control), and V1 (a virulent positive control) (4) constituted the control group (12-28 embryos each). Data analysis was performed using the Statistical Analysis System (SAS).

Results. The detection limit for direct detection of *E. sakazakii* on DFI agar was approximately 1000CFU/g of fecal matter while that on ESPM was 5000CFU/g of fecal sample. All *E. sakazakii* isolates were negative for all the virulence genes tested except for *ompA*. Among the test organisms, N72 (a bovine isolate) recorded the highest number of deaths while 52 (a bison isolate) had the lowest. For all three trials, more deaths were observed on day 2 and least on day 4 (data not shown). Based on the odd ratios, N72 was 63 times more likely to kill embryos than A4. This was followed by ATCC 29544 which was 47 times more likely to be lethal than A4.

Discussion. The present study is the first to report the isolation of *E sakazakii* from bovine and bison fecal samples. Muytjens and Kollee failed to recover *E. sakazakii* from bovine milk, cattle, domesticated animals, bird dung, soil and surface water in The Netherlands (5). This study demonstrates that the bovine and bison GI tracts could be possible reservoirs for *E. sakazakii*. The ATCC isolates and those recovered from ruminants were positive for *ompA* which constitutes the major outer membrane protein in gram-negative bacteria. It is also a major virulence factor in gram-negative pathogenic bacteria and has been associated with sepsis and meningitis. The virulence factors used in this study is known to be associated with extra intestinal *E.coli* infections (3). Since the majority of the primers were designed from *E. coli* pathogens, it is possible that similar genes found in *E. sakazakii* would not carry the same sequence. It should be noted that ELA serves as a preliminary study to assess the virulence of *E. sakazakii*. Further molecular analyses need be conducted in order to confirm these results and to further elucidate *E. sakazakii* pathogenesis.

References:

1. Restaino L, Frampton E, Linoberg W, Becker J. *J. Food Prot.* 69, 315-322, 2005.
2. Iversen C, Druggan P, Forsythe S. *Int. J. food Microbiol.* 96, 133-139, 2004.
3. Keller R, Pedroso M, Ritchman R, Silva R. *Infect. Immun.* 66, 645-649, 1998.
4. Gibbs PS, Mauer J, Nolan L, Wooley R. *Avian Dis.* 47,370-379, 2003.
5. Mutjens H, Kollee L. *Pediatr. Infect. Dis. J.* 9, 372-372, 1990.

FIBRONECTIN FRAGMENTS EXPOSE THE EVIL SIDE OF INTEGRIN SIGNALING AND ENHANCE OSTEOARTHRITIC DAMAGE

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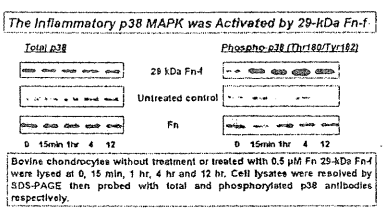
Introduction: According to the Arthritis Foundation, osteoarthritis (OA) affects approximately 30 million people in the United States and is also the leading cause of disability. Deterioration of cartilage in the joint causes OA. However, the mechanism of deterioration is unknown. Our laboratory has reported that fibronectin fragments (Fn-f), the degradation products of an extracellular matrix protein-fibronectin (Fn), can greatly augment matrix proteoglycan (PG) degradation via up-regulating matrix metalloproteinase (MMP) production. Among the Fn-fs derived from Fn, the N-terminal 29-kDa Fn-f is the most powerful in causing cartilage damage. Since native Fn is inactive in cartilage damage and is beneficial for growth of the major cells in cartilage, chondrocytes, it is fundamentally important to understand how Fn-fs drastically alter the normal Fn pathway and switch chondrocytes into a mode of hyper-catabolism. Our objectives were (1) to compare effects of the N-terminal 29-kDa Fn-f and Fn on alpha5beta1 integrin which is the major receptor for Fn; (2) to compare activation of three integrin associated kinases including focal adhesion kinase (FAK), proline-rich tyrosine kinase 2 (Pyk2) which is a soluble form of FAK, and Src kinase; (3) to compare activation of mitogen-activated protein kinases (MAPKs), which include ERK1/2, JNK, and p38, and activation of NF-kB, a transcription factor implicated in many inflammatory diseases; (4) to test whether inhibitors of the above kinases or NF-κB block Fn-f-induced MMP upregulation and PG depletion.

Methods: To investigate effects on alpha5beta1 integrin receptors, chondrocytes were isolated from the cartilage of bovine metacarpophalangeal joints and cultured in 10% serum / DMEM for 4-5 days then subjected to serum starvation for 24 hr. Cells were then treated with the 29-kDa Fn-f or Fn for 4 hr. After fixation with 3% paraformaldehyde, cells were permeabilized and probed with anti-alpha5 integrin subunit antibody. TRITC-labeled secondary antibody was used to visualize alpha5 integrin. Images were taken on a Zeiss confocal microscope. To investigate effects on activation of signaling factors, cells treated with Fn or Fn-f were lysed at 0, 15 min, 1 hr, 4 hr and 12 hr. Or cultures were adjusted to 10nM to 1 uM Fn or Fn-f and cell lysates were recovered at 1 hr. Western blotting was applied to display kinase activation via probing the blot with phospho-specific antibodies to signaling factors. To investigate effects of kinase or NF-kB inhibitors on MMP expression, cells were incubated with increasing concentrations of specific inhibitors 2 hr prior to addition of 29-kDa Fn-f. After 24 hr, media were recovered for MMP-3 or -13 blotting. The inhibitors tested were: AG-17 for Pyk2, Wortmannin for FAK, PP2 for Src, PD98059 for ERK1/2, SB202190 for p38, SP600125 for JNK and BAY11-7082 for NF-κB. To investigate effects of kinase inhibitors on cartilage damage, cartilage was preincubated with inhibitors for 24 hr, Fn-fs added and after 7 days in 10% serum/DMEM, PG content measured with DMB reagent.

Results: (1) The 29-kDa Fn-f caused disruption of alpha5beta1 integrin clustering seen in untreated cells and also enhanced internalization of the receptor while Fn had little effect; (2) Pyk2, Src, the three MAPKs and NF-kB were activated by 29-kDa Fn-f from 15 min to 12 hr with maximal effects mostly at 1 hr. Activation of these signaling factors was also elevated with increasing dose of Fn-f. However, Fn only activated FAK, ERK1/2 and had only short terms effects at 1 hr; (3) Inhibitors for Pyk2, three MAPKs and NF-kB down-regulated MMP-3, -13 expression as well as PG depletion induced by Fn-f. Although PP2 as a Src inhibitor did not decrease MMP expression, it reduced production of iNOS which is an inflammatory mediator and also elevated in Fn-f treated chondrocytes.



Bovine chondrocytes without treatment or treated with 0.5 μM Fn 29-kDa Fn-f were lysed at 0, 15 min, 1 hr, 4 hr and 12 hr. Cell lysates were resolved by SDS-PAGE then probed with total and phosphorylated p38 antibodies respectively.

Conclusion: We propose a tentative model of cartilage damage by Fn-f: after Fn-f binds to receptor, receptor aggregation is disrupted. This is associated with alternative activation of Pyk2 which is inactive in Fn treated cells. This leads to activation of MAPKs (p38, JNK and ERK1/2) and transcription factor NF-κB. Activated MAPKs and NF-κB would migrate into the nucleus, upregulate MMPs and cause consequent matrix proteoglycan (PG) degradation. In contrast, binding of native Fn does not alter receptor clustering, does not activate Pyk2 but does activate FAK, which normally involved in growth pathways, but not inflammatory pathways. Thus, with Fn treatment, the inflammatory MAP kinases-p38 and NF-κB are not activated and cartilage damage does not occur. Since the Fn-f system is a model of OA and represents a unique dysregulation of integrin signaling that may occur in other pathologies as well, further knowledge of the mechanism may be applicable to therapeutic intervention. Future work will more precisely delineate the mechanism.

HATs OFF TO TRANSCRIPTION

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In eukaryotes, DNA in the cell is associated with histone proteins to form nucleosomal arrays that are compacted into a highly ordered protein-DNA structure known as chromatin. Chromatin structure has been shown to act as a barrier to most biological processes that require access to DNA such as replication, transcription and DNA repair. Transcription is a critical biological process responsible for regulating the patterns of gene expression, which defines every living cell. Acetylation of histones by enzymes known as histone acetyltransferases (HAT) stimulates gene expression by relaxing chromatin structure, allowing access of transcription factors to DNA, whereas deacetylation of histones by histone deacetylases (HDACs) promotes chromatin condensation and transcriptional repression. It has also been proposed that acetylation of histones establishes a "code" that determines patterns that regulate gene expression. Our laboratory uses Simian Virus 40 (SV40), a small double-strand closed circular DNA genome as a model system to study chromatin modifications.

We have used various chromatin immunoprecipitation (ChIP) techniques followed by polymerase chain reaction (PCR) to determine the organization of a particular protein on the SV40 genome. We have determined the organization of hyperacetylated histone H4 and H3 in pooled SV40 chromosomes and observed that the organization of hyperacetylated H4 and H3 differed from each other and changed during the course of an infection. Depending upon the time post-infection, we observed patterns of organization consistent with active transcription, replication or chromatin remodeling within the promoter. We mapped the location of RNA Polymerase II (RNAPII) on transcribing SV40 chromosomes (operationally defined by the presence of the associated RNAPII which is responsible for transcription) during the course of infection and found that the reorganization of RNAPII on the SV40 genome occurs co-coordinately with the early to late transcriptional switch. We also determined the presence of hyperacetylated histones on transcribing chromosomes and found both histone H4 and H3 were associated with transcribing chromosomes.

In our recent study transcribing SV40 chromosomes were immune-selected with antibody to RNAPII and subjected to secondary chromatin immunoprecipitation with antibodies to hyperacetylated or unacetylated H4 or H3. Immune Selection Fragmentation and Immunoprecipitation (*ISFIP*) was used to determine the hyperacetylation status of histones independent of the location of the RNAPII and ReChromatin Immunoprecipitation (*ReChIP*) was used to determine their hyperacetylation status when associated with RNAPII. While hyperacetylated H4 and H3 were found in the coding regions regardless of the location of RNAPII, unacetylated H4 and H3 were only found at sites lacking RNAPII. The absence of unacetylated H4 and H3 at sites containing RNAPII was correlated with the specific association of the HAT, p300 with the RNAPII. In contrast, the presence of unacetylated H4 and H3 at sites lacking RNAPII was shown to result from the action of a HDAC based upon the effects of the inhibitor sodium butyrate. These results suggest that the extent of hyperacetylation of H4 and H3 during transcription alternates between hyperacetylation directed by an RNAPII associated HAT and deacetylation directed by an HDAC at other sites.

UPREGULATION OF TRPC1 FOLLOWING SERCA2 GENE SILENCING PROMOTES CELL SURVIVAL: A POTENTIAL ROLE FOR TRPC1 IN DARIER'S DISEASE.

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Ca^{2+} is a universal signaling molecule important for normal cellular functioning from cell growth to cell death. Any alteration in the Ca^{2+} homeostatic mechanism can abrogate a vast repertoire of regulated physiological events. Darier's disease (DD) is one uncommon anomaly of the skin wherein the keratinocytes are faced with deregulated Ca^{2+} signaling as a result of dominant mutations in the sarco/endoplasmic Ca^{2+} ATPase coding SERCA2 gene. The mechanism(s) involved in Ca^{2+} regulation in Darier's disease is not known. The present study was aimed to understand the mechanism of store-operated Ca^{2+} entry (SOCE) in this disease state. Transient receptor potential canonical-1(TRPC1) is known to function as a SOCE channel, so, we assessed the expression and function of TRPC1 in normal and altered skin cell physiology. Darier's disease patients demonstrated upregulation of TRPC1, but not TRPC3, in the squamous cell layers. Similar upregulation of TRPC1 was detected in epidermal layers of *SERCA2*$^{+/-}$ mice with a genetic makeup as in DD patients. Expression of SERCA2 si-RNA in a human keratinocyte cell line- HaCaT, increased TRPC1 levels and store operated Ca^{2+} influx; however, there was a decreased intracellular Ca^{2+} release in these cells, indicating the functional consequence of SERCA2 gene silencing. The TRPC1 mediated Ca^{2+} influx was blocked by SOCE inhibitors lanthanum and BTP2. Importantly, thapsigargin-induced apoptosis in HaCaT cells was attenuated by over expression of TRPC1 or SERCA2-siRNA, suggesting, keratinocytes in DD are resistant to apoptotic stimuli and hence have a hyperproliferative response. Isotretinoin (a drug used to suppress Darier's disease symptoms) suppressed Ca^{2+} entry and decreased survival of HaCaT cells. In conclusion, our study demonstrates upregulation of TRPC1 in Darier's disease. The elevated levels of TRPC1 increase the cytosolic Ca^{2+} influx thereby expressing a compensatory activity in response to the altered Ca^{2+} homeostasis. Of prime physiological importance, this rise in cytosolic Ca^{2+} is anti-apoptotic and drives cell proliferation. We suggest that the anti-apoptotic effect of TRPC1 can potentially contribute to abnormal keratosis in Darier's disease thus, establishing a novel link in skin cell physiology.

This work was supported by grants awarded to Brij B Singh (NSF, and NIH- 5P20RR017699).

SCHEDULE OF PRESENTATIONS — Auditorium — VCSU Science Center (Room 128)

Professional Communications – Session A – Heidi Super - Moderator

8:40 a.m. - TARGETING *MLL* FUSION GENES USING RNA INTERFERENCE
Heidi Super* and Cheryl Lepp, *Department of Biology, Minot State University*

9:00 a.m. - ANTI-APOPTOTIC REGULATOR HSP27 CONFERS CYTOPROTECTIVE EFFECTS THROUGH β-ADRENERGIC RECEPTOR-MEDIATED COMPLEX FORMATION WITH β-ARRESTIN.
Erin B. Harmon*, InSu Hahn[§], Bryon D. Grove[¶], Masaru Miyagi[§], and James E. Porter, *Departments of Pharmacology, Physiology, and Therapeutics, Biochemistry and Molecular Biology[§], and Anatomy and Cell Biology[¶], University of North Dakota*

9:20 a.m. - FLHD/FLHC AND FLIA AFFECT VIRULENCE OF *YERSINIA ENTEROCOLITICA* IN A CHICKEN EMBRYO MODEL. Birgit M. Prüß*, Shelley M. Horne, and Penelope S. Gibbs, *Department of Veterinary and Microbiological Sciences, North Dakota State University*

9:40 a.m. - MICROARRAY ANALYSIS IDENTIFIES KERATIN LOCI AS SENSITIVE BIOMARKERS FOR THYROID HORMONE DISRUPTION IN SALAMANDERS (*AMBYSTOMA*)
Robert B. Page, James R. Monaghan, Amy K. Samuels, Jeramiah J. Smith, Christopher K. Beachy*[§], and S. Randal Voss, *Department of Biology & Spinal Cord and Brain Injury Research Center, University of Kentucky, Lexington, KY, and [§]Department of Biology & Amphibian Growth Project, Minot State University*

10:20 a.m. – 11:00 a.m. Break, Refreshments will are available on the first floor of the Science Center.

Professional Communications – Session B – Jon Jackson - Moderator

1:00 p.m. STRATIGRAPHY OF NONMARINE MOLLUSCA FROM THE K/T BOUNDARY INTERVAL ON THE DECCAN PLATEAU, INDIA. Joseph H. Hartman[1]*, Dhananjay M. Mohabey[2], Henning Scholz[3], Sunil Bajpai[4], Marron Bingle[1], and Ritu Sharma[4], *[1]Department of Geology and Geological Engineering, University of North Dakota, Grand Forks, ND, [2]Geological Survey of India, Palaeontology Division, Nagpur, India; [3]Humbold-Univerität zu Berlin, Museum für Naturkunde, Institut für Paläeontologie, Berlin, Germany; [4]Indian Institute of Technology, Department of Earth Sciences, Uttaranchal, India*

1:20 p.m. - SOME ALTERNATIVES TO THE ARC-SINE SQUARE ROOT TRANFORMATION OF BINOMIAL DATA IN TWO-FACTOR DESIGNS
Jeff A. Morel, Department of Mathematics, Jamestown College, Jamestown, ND

1:40 p. m. - ERIONITE IN NORTH DAKOTA: A GEOLOGICAL HEALTH ALERT. Nels F. Forsman*, *Department of Geology and Geological Engineering, University of North Dakota*

2:00 p.m. Break, Refreshments will are available on the first floor of the Science Center.

Wetlands Symposium – Andre DeLorme - Moderator

3:30 p.m. - THE PLAINS CO_2 REDUCTION (PCOR) PARTNERSHIP – DEVELOPING CO_2 SEQUESTRATION OPPORTUNITIES FOR THE CENTRAL INTERIOR OF NORTH AMERICA
Barry W. Botnen*, Edward N. Steadman, John A. Harju, David W. Fischer[§], Lisa S. Botnen, Daniel J. Daly, Melanie D. Jensen, Erin M. O'Leary, Steven A. Smith, James A. Sorensen, and Charles R. Nelson
University of North Dakota Energy & Environmental Research Center, Grand Forks, ND, and [§]Fischer Oil & Gas, Inc., 5749 83rd Street South, Grand Forks

3:50 p.m. – CARBON SEQUESTRATION PROJECT, ND FARMERS UNION; HOW PRODUCERS MIGHT BENEFIT. Dale Enerson*, *North Dakota Farmers Union, Jamestown, North Dakota*

4:10 p.m. - SPECIES COMPOSITION OF A WETLAND PLANT COMMUNITY IN RELATION TO DISTURBANCE
Edward S. DeKeyser*, Mario Biondini, and Donald R. Kirby, *Department of Animal and Range Sciences, North Dakota State University*