# PROCEEDINGS OF THE NORTH DAKOTA ACADEMY OF SCIENCE

Volume 63 April 2009

#### NORTH DAKOTA ACADEMY OF SCIENCE

(Official State Academy; Founded: December 1908)

2009-2010

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#### **EDITOR**

101<sup>th</sup> Annual Meeting

April 30, 2009

Fargo, North Dakota

#### EDITOR'S NOTES

#### HISTORY

The *Proceedings of the North Dakota Academy of Science* (NDAS) was first published in 1948, with Volume I reporting the business and scientific papers presented for the 40th annual meeting, May 2-3, 1947. Through Volume XXI, the single yearly issue of the *Proceedings* included both abstracts and full papers. Commencing with Volume XXII, the *Proceedings* was published in two parts: A, published prior to the annual meeting, contained an abstract of each paper to be presented at the meeting, and B, published later, contained full papers by some of the presenters.

In 1979 (Vol. 33) the *Proceedings* changed to an  $8\frac{1}{2}$  x 11-inch format. Produced from camera-ready copy submitted by authors, it was distributed at the annual meeting. Commencing with Vol. 51 submissions were on computer disk; the *Proceedings* was then assembled with desktop publishing software. This approach allows the Editor to format papers so as to assure the *Proceedings* a consistent look. This method also produces an electronic copy of the *Proceedings*; the Secretary-Treasurer has the capability to generate electronic copies of past issues.

#### **VOLUME 62 ORGANIZATION**

In 2003 the NDAS council voted to accept all abstracts scheduled for presentation at the Annual Meeting. Thus, communications in volumes 58 to present haven't undergone a "typical" peer review. Rather, they provide an accurate reflection of the material presented before the NDAS membership at the Annual Meeting. The presentations in this year's *Proceedings* are presented in three major sections. The first contains the undergraduate communications presented as part of the A. Rodger Denison Student Research Competition. The second section comprises the graduate Denison Competition papers, and the final section comprises professional communications presented by faculty members of the Academy. Readers may locate communications by looking within the major sections of these *Proceedings* (see table of contents) or by referring to the author index on page 91

#### SYMPOSIA COMMUNICATIONS

Commencing with the 88th Annual Meeting [Vol. 50], Symposia presenters at annual meetings have had opportunity to contribute full-length articles or multiple-page contributions, thus providing much greater depth and coverage than that ordinarily possible. Speakers have presented educationally-oriented lectures and workshop discussions, and have still provided rigorous, more technical professional papers to the *Proceedings*.

#### COLLEGIATE AND PROFESSIONAL COMMUNICATIONS

Each Collegiate and Professional presentation at the annual meeting is represented by a Communication. Designed as more than a typical abstract but less than a full paper. Communications report results and conclusions, and permit sharing of important data and conclusions. Crucially, they provide for timeliness and ease of production.

#### CONSTITUTION AND BYLAWS

This issue of the Proceedings also contains the Constitution and Bylaws of the Academy, a list officers and committee members. We're working on maintaining a list of dues-paying members of the Academy (we'd appreciate your help in building and adding to this list with names of new and prospective members), a listing of past presidents of the Academy, and an index of presenters and paper authors. Copies of the financial statement and the unapproved minutes from last year's annual business meeting will be available at the business meeting.

#### IN APPRECIATION

The Academy wishes to acknowledge current and emeritus members of the Academy who continue to support the mission of the North Dakota Academy of Science Research Foundation through their special gifts. A listing of these supporters will accompany the Financial Report. The Academy also wishes to express its thanks to the presenters of papers at the Annual meeting, the session chairs, as well as all who have helped in organizing spaces and places, soliciting manuscripts, and compiling of this year's communications. The President of the Academy also wishes to sincerely thank Steven Kunkel who served as honored guest speaker at this year's meeting.

Birgit M Prüß President

Siegfried Detke Secretary-Treasurer, *Proceedings* Editor

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#### UNDERGRADUATE COMMUNICATIONS

IN THE

#### A ROGER DENISON COMPETITION

#### SCHEDULE OF PRESENTATIONS - UNDERGRADUATE SESSION #1

*Undergraduate session talks will be in the Hidatsa room in the Memorial Union – session will be chaired by A. Rodger Denison Competition judges* 

#### MORNING SESSION

- 7:30 Registration desk in the Legacy Lounge in the Memorial Union open
- 8:00 Greetings from President Prüß in the Century Theatre in the Memorial Union
- 8:20 ASSOCIATION BETWEEN PRESENCE OF INTEGRON-1 AND ANTIMICROBIAL RESISTANCE IN *SALMONELLA* ISOLATED FROM ANIMALS IN THE MIDWESTERN UNITED STATES. Galuak Aja, Sumit Arora, Susan Olet, Dawn Doetkott, Margaret L. Khaitsa. [p. 8]
- 8:40 ANALYSIS OF CHROMATIN STRUCTURE IN THE MYELOID-LYMPHOID LEUKEMIA GENE TRANSLOCATION BREAKPOINT CLUSTER REGION. Jeremy C. Horrell, Aileen M. Aldrich, Alysa L. Anderson, Heidi J. Super [p. 29]
- 9:00 MUTATION OF AN ASPARAGINE IN THE FIRST TRANSMEMBRANE DOMAIN OF THE HUMAN SEROTONIN TRANSPORTER RESULTS IN ENERGETIC UNCOUPLING OF NA FROM SEROTONIN TRANSPORT. Nathan Burbach, Patrick Lamb, Craig Lacher, Kristin Pavlish and L. Keith Henry [p. 11]
- 9:20 NUTRITIONAL IMPACTS ON MAMMARY GLAND VASCULARITY IN THE LACTATING EWE. Camille M. Jorgenson, Pawel P. Borowicz, Joel S. Caton, Dale A. Redmer, Lawrence P Reynolds, and Kimberly A. Vonnahme [p.12]
- 9:40 USING SPECIES-SPECIFIC DIFFERENCES OF THE HUMAN AND *C. ELEGANS* SEROTONIN TRANSPORTERS TO DEFINE MOLECULAR INTERACTIONS FOR ANTIDEPRESSANT, COCAINE AND AMPHETAMINE BINDING. Patrick Lamb, Kristin Pavlish and L. Keith Henry [p.15]
- 10:00 ANTIMICROBIAL RESISTANCE PATTERNS AND PRESENCE OF CLASS 1 INTEGRONS IN *E. coli* ISOLATED FROM RAW AND READY TO EAT TURKEY MEAT. Lisa R. Mowry, Susan Olet, Dawn K. Doetkott, Margaret L. Khaitsa. [p.16]
- 10: 20 BREAK (refreshments and exhibits in the Plains Room in the Memorial Union)
- 10:40 PLACENTAL TISSUE mRNA EXPRESSION OF ANGIOGENIC FACTORS (AF) AND THEIR RECEPTORS (AFR) IN RESPONSE TO IN VITRO HYPOXIA AS INFLUENCED BY MATERNAL NUTRITION IN ADOLESCENT SHEEP AT DAY 75 OF PREGNANCY. Mahalakshmi Razdan, Raymond P. Aitken, John S. Milne, David B. Carlson, Lawrence P. Reynolds, Jacqueline M. Wallace, Anna T. Grazul-Bilska, Dale A. Redmer, and Mary Lynn Johnson [p.17]
- 11:00 TRANSCRIPTIONAL CHANGES IN *NEISSERIA MENINGITIDIS* IN A CELL-EXCLUSIONCO-CULTURE WITH A *NEISSERIA LACTAMICA* BIOFILM. Ritter, Alex; Compton, Kylene; Drees, Jeremy; Newton, Casey; Strand, Krystle and Aho, Ellen [p.18]

- 11:20 INFLUENCE OF MATERNAL NUTRITION ON FETAL PLACENTAL VASCULARITY IN ADOLESCENT SHEEP AT DAY 75 OF PREGNANCY. Anuradha Sakhuja, Raymond P. Aitken, John S. Milne, Pawel Borowicz, Larry P. Reynolds, Anna T Grazul-Bilska, Dale A. Redmer and Jacqueline M. Wallace. [p.19]
- 11:40 VASCULAR GROWTH IN UTERINE TISSUES DURING EARLY PREGNANCY IN SHEEP. Robert Wroblewski, Pawel P Borowicz, Dale A Redmer, Lawrence P Reynolds and Anna T Grazul-Bilska. [p.26]
- 12:00 LUNCH (served in the Plains Room in the Memorial Union). We will also conduct our business meeting (open to all members) during the lunch hour.

#### AFTERNOON SESSION

- 1:00 EFFECTS OF NUTRIENT RESTRICTION AND DIETARY SELENIUM ON EXPRESSION OF GAP JUNCTIONAL PROTEIN CONNEXIN (CX) 43 IN FETAL OVARIES OBTAINED FROM SHEEP IN LATE PREGNANCY; IMPLICATIONS FOR DEVELOPMENTAL PROGRAMMING. Dheeraj Soni, Samantha Billings, Kimberly A. Vonnahme, Jerzy Bilski, Joel S. Caton, Dale A. Redmer, Lawrence P. Reynolds and Anna T. Grazul-Bilska . [p.22]
- 1:20 BACTERIAL POPULATION STRUCTURE OF A GASIFICATION COOLING TOWER. Joshua J. Sweet, Brian Striefel, Paul W. Lepp [p.23]
- 1:40 EXAMINING THE ENVIRONMENTAL AND GENETIC CONTROL OF *ESCHERICHIA COLI* BIOFILM FORMATION. Karan Verma, Anne Denton, Birgit Prüβ [p.24]
- 2:00 THE 5TM VPAC1 ISOFORM BLOCKS CAMP SIGNALING EVOKED FROM THE FULL-LENGTH 7TM RECEPTOR. Erich Raymond Wilkerson, Rebecca J Hermann, Donald R Branch, Glenn Dorsam. [p.25]
- 2:20 ANTIMICROBIAL RESISTANCE PATTERNS OF SALMONELLA ISOLATED FROM SICK ANIMALS . Skye Smith, Jennifer Nguyen, Susan Olet, Dawn Doetkott, Margaret L. Khaitsa [p.21]
- 2:40 BREAK (refreshments and exhibits in Plains Room in Memorial Union)
- 3:00 RAPID SYNTHESIS OF N-[1-(2,4-DICHLOROPHENYL)ETHYL]FORMAMIDE Mikhail M. Bobylev and Kurt Bowen. [p.9]
- 3:20 APPLICATION OF THE ACCELERATED LEUCKART REACTION TO SUBSTITUTED BENZALDEHYDES. Mikhail M. Bobylev and Steven Lewis. [p.13]
- 3:40 SMALL IS DIFFERENT HOW NANOSCIENCE CAN HELP TO DEVELOP CATALYSTS FOR CLEANER FUELS. M. Komarneni, A. Sand, J. Goering, U. Burghaus . [p.14]
- 4:00 SYNTHESIS OF BENZHYDRYLFORMAMIDES VIA THE ACCELERATED LEUCKART REACTION. Mikhail M. Bobylev and Tanner Scofield. [p.20]
- 4:20 RAPID SYNTHESIS OF PIPERONYLFORMAMIDE. Mikhail M. Bobylev and Zane Z. Young. [p.27]

## 4:40 OPTIMIZATION OF GOLD NANOPARTICLE SIZE FOR USE IN LATERAL FLOW BIOSENSORS, Kristen Keller, Xun Mao, Guodong Liu [p.28]

#### **EVENING**

6:00 Banquet will be in the Plains Room in the Memorial Union

### ASSOCIATION BETWEEN PRESENCE OF INTEGRON-1 AND ANTIMICROBIAL RESISTANCE IN SALMONELLA ISOLATED FROM ANIMALS IN THE MIDWESTERN UNITED STATES.

Galuak Aja\*, Sumit Arora, Susan Olet, Dawn Doetkott, Margaret L. Khaitsa.

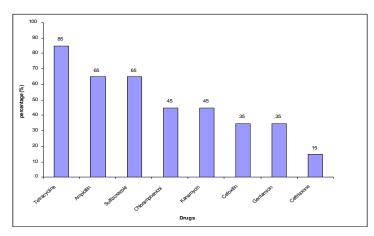
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The ability of microorganisms to evade or to become resistant to antibiotics can be acquired through integrons, genes that consist of a central variable region that often harbors antibiotic-resistance gene cassettes (White et al, 2001). Integrons are common in many different types of bacteria including Salmonella spp. Moreover antimicrobial resistance is becoming an increasing threat (Ray et al, 2007). The objectives of this study were 1) to investigate Antimicrobial Resistance (AMR) patterns of Salmonella isolated from sick animals in the Midwestern United States (ND, MN, MT) 2) determine presence of integron1 in the Salmonella isolates and 3) determine if there is an association between AMR and presence of integron1. AMR testing was done using a NARMS panel of 15 antimicrobials as per the manufacturer's instructions (Sensitire, Trek Diagnostics System, and Westlake, Ohio). Screening for the class 1 integrons was done using PCR with primers specific for the int1. Results were entered into an excel spread sheet and descriptive statistics run using EPI INFO version 3.2. Chi square analysis was used to determine association between AMR and presence of integron1. The % resistance of Salmonella isolates to antimicrobials ranged from 0% (Ciprofloxacin, Nalidixic Acid, Amikacin and Trimethoprim/Sulph) to 71% for Tetracycline. Ceftiofur was non-interpretable. Of 54 Salmonella isolates tested, integron 1 was present in 20 (37%) of the isolates and the majority (85%) of these isolates were resistant to tetracycline (Figure 1). At univariate analysis, preliminary results indicated that presence of integron 1 was significantly associated with AMR to only gentamycin (OR=5.56; 95% CI=1.24, 24.93; p=0.0225) (Table 1). Results of multiple logistic regression are pending. Preliminary results indicate widespread AMR among the Salmonella tested. and presence of additional mechanisms for AMR transfer in addition to integrons. These mechanisms warrant further investigation.

Table 1: Chi-square results of association between presence of ir	ntegron 1 on Salmonella and AMR (n=54	)
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Drug	Chi square	Fisher's Exact p-value	Odd Ratio (OR)	95% CI
Amo/ClA	0.175	0.445	1.2667	0.42, 3.83
Ampicillin	0.4336	0.264	1.4662	0.47, 4.59
Cefoxitin	0.1645	0.457	0.787	0.25, 2.51
Ceftriaxone	-	0.227	0.451	0.11, 1.92
Chloramphenicol	0.9678	0.241	0.5727	0.19, 1.75
Gentamicin	-	0.0225	5.5641	1.24, 24.93
Kanamycin	0.0965	0.381	1.1958	0.39, 3.70
Sulfizoxazole	0.0012		1.0214	0.31, 3.31
Tetracycline	2.65	0.096	3.6957	0.72, 19.10

Figure 1: Distribution of AMR in Salmonella isolates tested for presence of integron1



#### References

Ray, K.A., et al. (2007). Presence of Antimicrobial Resistance among *Salmonella* on midwest and northeast USA dairy farms. Preventative Veterinary Medicine. 79:204-223

White, P. A., et al. (2001). Integrons and gene cassettes in the *Enterobacteriaceae*. *Antimicrob*. *Agents Chemother*. 45:2658-2661. [PubMed].

#### RAPID SYNTHESIS OF N-[1-(2,4-DICHLOROPHENYL)ETHYL|FORMAMIDE

Mikhail M. Bobylev and Kurt Bowen\*

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N-[1-(2,4-dichlorophenyl)ethyl]formamide (I) is an important intermediate in the synthesis of biologically active compounds, including agrochemicals and pharmaceuticals. I can be synthesized from 2,4-dichloroacetophenone by the Leuckart reaction. In the literature, the synthesis of I was described only once, and it appeared to be a very slow reaction, taking 5 hours to complete. Recently, we developed an accelerated procedure for the synthesis of formamide fungicides. In this work, the accelerated procedure was successfully applied to the synthesis of I. The reaction was fully completed in 1 minute and produced I in good yield. The new reaction opens the way for the fast synthesis of I and its derivatives in the laboratory practice and industry. The project is supported by NIH grant P20 RR016741 from the NCRR

### A NOVEL DOWN-STREAM TARGET OF MYOSTATIN: IMPORTANT IN MUSCLE GROWTH AND REGULATION?

Elizabeth B. Braschayko, Nicholas J. Galt & Peggy R. Biga

#### North Dakota State University, Fargo, ND

Myostatin (MSTN) negatively regulates muscle growth in mammals, with some evidence in support of a similar role in fish. MSTN levels have been shown to be down-regulated in response to growth hormone and up-regulated during fasting in both giant danio (*Danio aequipinnatus*) and rainbow trout (*Oncorhynchus mykiss*). These data support a role of MSTN in fish growth. The intent of this project was to isolate and identify a potential downstream target of MSTN in muscle tissue. By utilizing the zebrafish (*Danio rerio*) genome and rainbow trout EST databases, a novel target, *akirin* was virtually cloned from rainbow trout and zebrafish. Utilizing degenerate primers, we subsequently isolated, empirically cloned, and sequenced *akirin* from the giant danio and rainbow trout. Semi-quantitative and real-time PCR demonstrate similar tissue distribution between akirin and MSTN gene expression in adult giant danio. In addition, it has been demonstrated that akirin gene expression is down-regulated during fasting, while MSTN is up-regulated, suggesting an interaction in regulation of growth and metabolism. We hypothesize a regulatory interaction between MSTN and akirin, with opposing growth functions.

# MUTATION OF AN ASPARAGINE IN THE FIRST TRANSMEMBRANE DOMAIN OF THE HUMAN SEROTONIN TRANSPORTER RESULTS IN ENERGETIC UNCOUPLING OF NA FROM SEROTONIN TRANSPORT.

Nathan Burbach<sup>1\*</sup>, Patrick Lamb<sup>1</sup>, Craig Lacher<sup>2</sup>, Kristin Pavlish<sup>1</sup> and L. Keith Henry<sup>1</sup>

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The serotonin transporter (SERT) like the norepinephrine (NET) and dopamine (DAT) transporters is a member of the Na and Cl-dependent SLC6 transporter family. Serotonin (5-HT) transport is coupled to the extracellular Na and Cl gradients allowing concentrative re-uptake of 5-HT. Previously we identified an aspargine residue (N101) in transmembrane domain 1 of SERT that upon replacement with alanine or cysteine renders the transporter Cl-independent. The study presented here examines the impact of mutations at N101 on the requirement for Na. The human SERT N101A and N101C mutants were transiently expressed in HEK-293 MSR cells and assayed for [3H]5-HT transport under Na-free and Na-reduced conditions (replaced with N-Methyl-D-Glucamine (NMDG)). In contrast to native hSERT, the N101A and N101C mutants display dose dependent modulation of 5-HT transport in Na-free conditions. Preliminary assays suggest the N101 mutants do not alter cation specificity as Li cannot replace Na to support 5-HT uptake in the N101A or N101C mutants. Additionally, site-directed mutants were generated at the residue homologous to hSERT N101 in C. elegans SERT (N122) and DAT (N82) to evaluate the functional conservation of this residue in ion dependence. [3H]5-HT transport assays with MOD-5 N122A and N122C mutants show the substitutions are functionally tolerated. Ion dependence assays are underway. Our findings represent the first identification of a residue in monoamine neurotransmitter transporters whose substitution results in the partial or complete uncoupling of ion binding from substrate translocation and may lead to a fundamental understanding as to how the drug "ecstasy" and the interacting protein syntaxin 1A modulate SERT function

### NUTRITIONAL IMPACTS ON MAMMARY GLAND VASCULARITY IN THE LACTATING EWE

Camille M. Jorgenson\*, Pawel P. Borowicz, Joel S. Caton, Dale A. Redmer, Lawrence P Reynolds, and Kimberly A. Vonnahme

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Previously, our laboratory demonstrated that both under- and over-nutrition during mid to late gestation negatively impacts colostrum production. Moreover, the immunoglobulin G (IgG) concentration in the colostrum was also reduced in both under- and over-nourished ewes compared to control ewes. Immunoglobulins are important as this provides passive immunity to the neonate. Since blood flow to the mammary gland directly impacts milk production, we hypothesized that vascularity of the mammary gland would be reduced if milk production is reduced. How maternal nutrition during gestation impacts capillary numbers, capillary surface area, capillary area or capillary size in the developing mammary gland is unknown. In the current study, ewes were fed either Control diets (100% of NRC requirements for gestating ewes), Low diets (60% of Control), or High diets (140% of control) from day 50 of gestation until lambing. Mammary glands were collected and vascularity measures were determined. Vascular development was studied at the level of the alveoli. Mammary gland sections were perfusion fixed with Carnov's solution, sectioned at 4 µm, stained with the immunohistochemistry for Factor VIII, a specific endothelial cell marker. Photomicrographs were taken with 400x magnification using a Nikon Eclipse E800 microscope equipped with Nikon DXM 1200F digital camera (n=10 pictures per slide, 85734.7 µm2 per picture). Vascularity was determined for mammary tissue with the following measurements taken: the cross-sectional capillary area density (CAD, total capillary area as a proportion of tissue area), capillary number density (CND, total number of capillaries per unit of tissue area), and capillary surface density (CSD, total capillary circumference per unit of tissue area). To provide a measure of average capillary size, we also calculated the average cross-sectional area per capillary (APC) by dividing the CAD by the CND. The images were analyzed for vascular microanatomy using the Image-Pro Plus image analyzes software. There was no nutritional effect on CAD (P = 0.23) or on APC (P = 0.21). However, diet did impact the CSD (P = 0.05) with Low and High fed ewes having decreased capillary surface area compared to Control ewes (61.96 and 56.66 vs.  $73.40 \pm 4.43$  microns, for Low, High, and Control ewes, respectively). High ewes also tended (P = 0.09) to have a reduction in CND compared with Control ewes with Low ewes being intermediate. Reductions in mammary capillary numbers and nutrient exchange area may have directly impacted the volume and composition of the colostrum that we have previously reported.

This project was partially supported by National Research Initiative Competitive Grants no. 2005-35206-15281 from the USDA Cooperative State Research, Education and Extension Service; and by the Ronald E. McNair post baccalaureate Achievement Program.

### APPLICATION OF THE ACCELERATED LEUCKART REACTION TO SUBSTITUTED BENZALDEHYDES

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Aldehydes and ketones are valuable building blocks for chemical industry. Reductive amination is a fundamental chemistry process that dramatically expands the application of aldehydes and ketones by transforming them into amines. The Leuckart reaction is a unique one step method of reductive amination. It is a remarkably simple process that includes only two components: the carbonyl compound and formamide. The reaction is completed simply by heating the components at 160°C to 185°C for 6 to 25 hours. The long processing time seems to be the only shortcoming of the reaction. During their work with formamide fungicides, Bobylev et al developed an accelerated procedure for the Leuckart reaction. The accelerated Leuckart reaction could be completed in 30 minutes or less. As a highly intensive process, the accelerated Leuckart reaction has a potential of being successful in the areas where the traditional Leuckart reaction was not. Specifically, it was believed that the Leuckart reaction does not work well on substituted benzaldehydes and that certain substituted benzylformamides cannot be obtained via the Leuckart reaction. In this work, the accelerated Leuckart reaction was successfully applied to 4-chlorobenzaldehyde and produced 4-chlorobenzylformamide in good yield. The project is supported by NIH grant P20 RR016741 from the NCRR.

### SMALL IS DIFFERENT - HOW NANOSCIENCE CAN HELP TO DEVELOP CATALYSTS FOR CLEANER FUELS

#### M. Komarneni, A. Sand\*, J. Goering, U. Burghaus

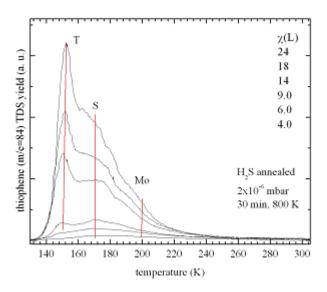
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and

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Due to the importance of the hydrodisulfurization (HDS) of compounds for applications in the refinement of fuels and generation of chemical feedstock, HDS has been the focus of much research over the past few decades. The catalytic activity of two novel HDS catalysts, consisting of either MoS<sub>2</sub> or WS<sub>2</sub> fullerene-like nanoparticles (supported on silica or sapphire) has been characterized using kinetics and spectroscopic measuring techniques. Thiophene was used as a probe molecule since it is the smallest sulfur compound present in crude oil. Besides molecular adsorption of thiophene at low temperatures, the formation of alkanes, as desulfurization

products, was detected at greater temperatures when keeping the catalysts in hydrogen ambient. Further, samples were annealed in hydrogen, oxygen, and hydrogen sulfide ambients, subsequent and HDS experimentation showed the reduced samples exhibited greater HDS activity than fully sulfided samples. Interestingly, on MoS<sub>2</sub> nanoparticles, the Mo and S adsorption sites distinguished via could kinetics experiments (thermal desorption spectroscopy) spectroscopic data (Auger electron spectroscopy), which may act as the active sites for HDS. Redhead analysis allowed for the calculation of thiophene binding energies for each sample.



Acknowledgements: Financial support was provided by the Chemical Sciences, Geosciences and Biosciences Division, Office of Basic Energy Sciences, Office of Science, U.S. Department of Energy

# USING SPECIES-SPECIFIC DIFFERENCES OF THE HUMAN AND *C. ELEGANS* SEROTONIN TRANSPORTERS TO DEFINE MOLECULAR INTERACTIONS FOR ANTIDEPRESSANT, COCAINE AND AMPHETAMINE BINDING.

#### Patrick Lamb\*, Kristin Pavlish and L. Keith Henry

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The serotonin neurotransmitter transporter (SERT) is found on the pre-synaptic neuron and in platelets and belongs to the NSS (neurotransmitter/Sodium Symporter) family. SERT is the major target of many antidepressants and drugs of abuse such as cocaine and ecstasy. However, despite its clinical relevance, the interaction of SERT with these important compounds is poorly understood at the molecular level. To identify residues involved in inhibitor binding to SERT, we are investigating species-specific differences in drug potency between the evolutionarily divergent human (hSERT) and C. elegans SERT (MOD-5) transporters. Using molecular techniques, we subcloned MOD-5 cDNA into the plasmid vector pcDNA3.1 for expression in mammalian tissue culture cells. Furthermore, MOD-5 was engineered to express V5 and HIS epitope tags on the c-terminus for use in immunoblotting and purification studies (MOD-5-VH). Addition of the c-terminal epitopes does not appear to alter transport activity as both MOD-5 and the MOD-5-VH proteins displayed comparable levels of transport activity when expressed in HEK-MSR cells. Expression of MOD-5-VH fusion protein was verified by Western blot analysis using anti-V5 monoclonal antibodies where ~64 and ~83 kDa bands were detected representing the multiple glycosylated forms of MOD-5-VH. [3H] serotonin competition uptake assays with antidepressant compounds such as paroxetine, imipramine, r-citalogram and scitalogram are currently underway to determine Ki values for various SERT-interacting compounds.

#### ANTIMICROBIAL RESISTANCE PATTERNS AND PRESENCE OF CLASS 1 INTEGRONS IN E. coli ISOLATED FROM RAW AND READY TO EAT TURKEY MEAT.

Lisa R. Mowry<sup>1, 2\*</sup>, Susan Olet<sup>2</sup> Dawn K. Doetkott<sup>2</sup>, Margaret L. Khaitsa<sup>2</sup>.

<sup>1</sup>Department of TRIO services, McNair Scholars Program, <sup>2</sup>Department of Veterinary and Microbiological Sciences, North Dakota State University, Fargo, ND 58105.

Antimicrobial resistance (AMR) in gram negative bacteria is associated with carriage of specific DNA elements called Class 1 integrons<sup>1</sup>. Class 1 integrons are commonly associated with resistance towards the antimicrobials Trimethoprim-Sulfamethoxazole, Gentamicin, Tetracyline, and Streptomycin. The objectives of this study are 1) determine AMR patterns of Escherichia coli isolates from raw and ready to eat (RTE) turkey meat; 2) examine the prevalence of class 1 integron in isolates; 3) determine the association between presence of class 1 integron and AMR. The E. coli isolates used in this study were isolated in an earlier study <sup>2</sup> from turkey meat products purchased from retail outlets in the Midwestern United States. All isolates were screened for AMR using full-range Minimum Inhibitory Concentration (MIC). PCR was used to test for presence of class 1 integron. A statistical measure of association between class 1 integron presence in E. coli and AMR was determined by odds ratio (OR) with 95% confidence interval (CI). A multiple logistic regression model was run to determine the group of antimicrobials that showed the best association between AMR and presence of integron 1 in the E. coli isolates. Of the 242 isolates tested, 102 (42%) of which 88/194 (45%) raw and 14/48 (29%) RTE were positive for integron 1. The results show significant association between class 1 integron presence and antimicrobial resistance towards, Amoxicillin/ Clavulanic Acid (OR 2.5 CI 1.49 - 4.27, p-value = 0.0005); Gentamicin (OR 11 CI 5.90 - 20.52, p-value <0.0001); Streptomycin (OR 3.4 CI 1.97 - 5.73, p-

value <0.0001); Sulfizoxazole (OR 5.4 CI 3.08 -9.35, p-value < 0.0001); Tetracycline (OR 7.1 CI 3.05 - 16.45, p-value < 0.0001). The percent of AMR ranges from 6% - 68% of the E. coli isolates possessing integron 1 (see Fig. 1) when exposed to multiple antimicrobials. Multiple logistic regression results indicated that presence of integron 1 was most significantly associated with AMR towards a combination of Gentamicin and Tetracycline. The sample size used for this model was 242; the deviance was used to assess the model fit (Deviance = 1) and the Hosmer and Lemeshow Goodness-to Fit test suggested the model was adequate  $\chi^2 = 2.18$  and p-value = 0.3354. Our study indicates widespread occurrence of AMR among, E. coli isolates from raw and RTE turkey meat from the Midwestern US.

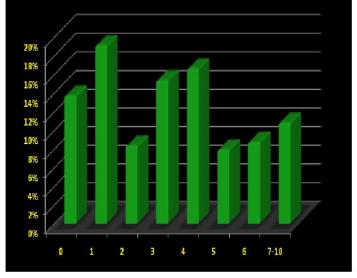


Fig. 1. Percent of 242 E. coli isolates displaying resistance toward multiple antimicrobials.

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# PLACENTAL TISSUE mRNA EXPRESSION OF ANGIOGENIC FACTORS (AF) AND THEIR RECEPTORS (AFR) IN RESPONSE TO IN VITRO HYPOXIA AS INFLUENCED BY MATERNAL NUTRITION IN ADOLESCENT SHEEP AT DAY 75 OF PREGNANCY

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Placental and fetal growth is severely compromised in adolescent ewes over nourished throughout gestation. Switching ewes from a high (H) to a moderate-control (C) intake at day 50 of gestation (length of pregnancy in sheep is ~145 days) prevents these negative pregnancy outcomes (1). However, recent analyses suggest that placental proliferation rate and vascularity are perturbed in high intake pregnancies as early as day 50. The aim of this study was to determine incubated placental explant AF/AFR mRNA response to hypoxia in C and H ewes at day 75, and in ewes switched from a H to a C intake (H-C) from day 50 until day 75.

Singleton pregnancies (single sire) were established by embryo transfer to maximize similarity between offspring. Whole placentomes (the part of the placenta where nutrient exchange occurs between the fetus and dam), were collected at day 75 of gestation and separated into fetal cotyledon (COT) and maternal caruncle (CAR) tissues. These tissue explants were incubated in normal oxygen concentration (20% O<sub>2</sub>) or hypoxic (5% O<sub>2</sub>) conditions for 24 hr and then frozen for RNA extraction followed by quantitative real-time RT-PCR determination of placental AF/AFR mRNA.

Maternal intake did not affect fetal or total placentome mass at day 75 in H or C groups; however, switching H ewes to C at d 50 increased placentome mass at day 75 (2). In COT, hypoxia increased (P<0.03) overall mRNA expression of vascular endothelial growth factor (VEGF) by 177  $\pm$  15%, placental growth factor (PIGF) by 195  $\pm$  14%, angiopoietin-2 (ANGPT2) by 180  $\pm$  11% and ANGPT receptor Tek by 130  $\pm$  6%, and decreased (P<0.01) expression of ANGPT1 to 79  $\pm$  5% compared with normal O<sub>2</sub>. In addition, hypoxia tended to increase VEGF receptor Flt1 (P<0.06, 139  $\pm$  9%), and tended to decrease VEGF receptor KDR (P=0.07; 82  $\pm$  4%) and endothelial nitric oxide receptor (NOS3; P<0.1, 87  $\pm$  7%) mRNA expression in COT explants. H or H-C maternal dietary intakes substantially reduced (P<0.004) hypoxia-induced increases in PIGF mRNA expression compared to C (175  $\pm$  11% in H, 157  $\pm$  17% in H-C, and 254  $\pm$  29% in C, where normoxic=100%) in COT. Significant effects of maternal nutrition or hypoxia on AP/APR mRNA expression in CAR explants were not observed.

In summary, hypoxia substantially affects mRNA expression of a variety of AF/AFR in placental COT explants. More importantly, H or H-C intakes known to alter placental and fetal growth may act through a reduction in to the COT response to hypoxia-induced expression of PIGF. Thus, these data emphasize the importance of adequate maternal nutrition in regulation of fetal and placental growth and development.

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### TRANSCRIPTIONAL CHANGES IN *NEISSERIA MENINGITIDIS* IN A CELL-EXCLUSION CO-CULTURE WITH A *NEISSERIA LACTAMICA* BIOFILM

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The genus *Neisseria* consists of pathogenic and non-pathogenic species of gram-negative diplococci. *N. meningitidis* is a cause of meningitis in humans, and *N. lactamica* is a commensal species that commonly inhabits the human nasopharynx. *N. meningitidis* enters the body through the nasopharynx where it may interact with biofilms of commensal bacteria growing on airway epithelial tissue. *Neisseria* species not only have the capacity to grow as biofilms, but also form microcolonies that display coordinated movement (1, 2). These multicellular behaviors make it reasonable to infer that *Neisseria* engage in cell-cell communication, but the molecules that mediate intercellular communication have not been well-characterized in *Neisseria*. Examination of the genomes of *N. lactamica* and *N. meningitidis* reveals only two genes, *luxR* and *luxS*, coding for known molecules involved in quorum sensing or other prokaryotic cell-cell signaling pathways. These genes are part of the well-characterized autoinducer-2 pathway, which plays a role in both intraspecies and interspecies communication in several prokaryotic organisms (3). Other known genes in this pathway, however, are absent from the sequenced neisserial genomes. The goal of this study was to investigate intercellular communication among *Neisseria* by measuring transcriptional changes in planktonic *N. meningitidis* in response to molecules produced by *N. lactamica* biofilms.

We have developed a model system in which cultures of planktonic *N. meningitidis* and biofilm-grown *N. lactamica* are separated by a 0.4 µm porous polycarbonate membrane that prohibits the passage of bacterial cells but permits the exchange of molecules across the membrane. We established 36-hour static biofilms of *Neisseria lactamica* strain NL4 in tissue culture wells and added membrane inserts holding planktonic, encapsulated *N. meningitidis* strain FAM18 to the wells. This cell-exclusion co-culture was incubated for two hours, and RNA was extracted from the planktonic *N. meningitidis*. We performed microarray analysis on the meningococcal RNA using single-color custom Affymetrix arrays containing probesets specific to the *N. meningitidis* genome. The transcription profile of the co-cultured meningococci was compared to that of meningococci grown in the same system in the absence of a *N. lactamica* biofilm.

N. meningitidis grown in the co-culture system displayed numerous transcriptional changes. The arrays contained probesets to 36,232 target sequences. Preliminary analyses indicate two-fold or greater changes in 412 (1.1%) of these targets (p < 0.05). Among the 412 differentially expressed sequences, 180 (43.7%) showed increased expression and 232 (56.3%) showed decreased expression. The variably expressed targets included 247 (60%) sequences of unknown function. Several of the sequences that were differentially expressed represent genes associated with meningococcal pathogenesis. These include genes encoding proteins involved in obtaining iron from the host (fbpC and lbpB); genes that code for proteins involved in the formation of pili, neisserial surface structures that are important for motility and attachment to host cells (pilC, pilT, pilQ, pilS, and pilW); and genes coding for additional adhesins (nadA, opa). The nadA gene is of particular interest because NadA is a component of a new N. meningitidis serogroup B vaccine currently in human trials. The data from this co-culture model system suggest that meningococci may undergo important transcriptional changes when they enter the human nasopharynx and encounter resident bacterial populations. Further studies will focus on both known genes associated with pathogenesis and unknown genes that may play a role in novel prokaryotic signaling pathways.

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### INFLUENCE OF MATERNAL NUTRITION ON FETAL PLACENTAL VASCULARITY IN ADOLESCENT SHEEP AT DAY 75 OF PREGNANCY.

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The nutrient transfer capacity of the placenta plays a central role in determining the prenatal growth trajectory of the fetus and influences birth weight and neonatal viability. Placental and fetal growths are severely compromised by late pregnancy in adolescent ewes (1,2). The aim of the present study was to investigate whether placental vascular growth and pregnancy outcome could be altered by switching adolescent dams from a high to a moderate nutrient intake at the end of the first trimester (Day 50).

Singleton pregnancies to a single sire were established by embryo transfer and thereafter adolescent dams were offered high (n=13) dietary intake. This level of diet was calculated to promote a rapid maternal growth rate. At day 50 of gestation (pregnancy in sheep=145d days), some dams (n=8) had their dietary intake switched to a moderate plane of nutrition, but the rest (n=5) remained at high dietary intake plane of nutrition. On day 75 of pregnancy, placentomes from each ewe were fixed with Carnoy's solution by perfusion of the main vessel supplying the cotyledonary (COT; fetal) tissue. After fixation, tissues were embedded in paraffin, sectioned, and stained with hematoxylin and periodic acid-Shiffs. Photomicrographs were taken at 400× magnification using a Nikon DXM 1200 digital camera (Fryer Company, Inc., Chicago, IL). Vascularity was then determined by image analysis (Image-Pro Plus, version 5.0; Media Cybernetics, Houston, TX). The following parameters were determined for each photomicrograph (n=10 per slide/ewe): tissue area, shrinkage area (the effect of fixation that was subtracted from the tissue area), cross-sectional capillary area density (CAD, total capillary area as a proportion of tissue area), capillary number density (CND, total number of capillaries per unit of tissue area), and capillary surface density (CSD, total capillary circumference per unit of tissue area). To provide a measure of average capillary size, the average cross-sectional area per capillary APC was calculated by dividing the CAD by the CND.

Maternal dietary intake affected (P<0.04) fetal cotyledon CND number but not other measurements of vascularity. CND was less (P < 0.04) in placentomes of ewes fed moderate diet compared with ewes fed high nutrition level ( $346\pm22$  vs.  $289\pm14$ ).

Thus, reducing maternal dietary intake from a high to a moderate level at the end of the first trimester (day 50) seems to stimulate placental vascular growth and may play a role in enhancing pregnancy outcome.

Supported by NIH grants HL64141 to LPR and DAR, and P20 RR016741 from the INBRE program of the National Center for Research Resources.

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### SYNTHESIS OF BENZHYDRYLFORMAMIDES VIA THE ACCELERATED LEUCKART REACTION

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Benzhydrylamines are important intermediates in the synthesis of biologically active compounds, including agrochemicals and pharmaceuticals. Benzhydrylamines can be synthesized from benzophenones by the Leuckart reaction via the intermediate benzhydrylformamides. The synthesis of benzhydrylformamides via the Leuckart reaction is a very slow procedure, taking several hours to complete. Recently, we developed an accelerated procedure for the synthesis of formamide fungicides via the Leuckart reaction. In this work, the accelerated procedure was successfully applied to the synthesis of benzhydrylformamides from benzophenones. The reaction is fully completed in 15-30 minutes and produces benzhydrylformamides in quantitative yield. The new reaction opens the way for the fast synthesis of benzhydrylamines and their derivatives in the laboratory practice and industry. The project is supported by NIH grant P20 RR016741 from the NCRR.

#### ANTIMICROBIAL RESISTANCE PATTERNS OF SALMONELLA ISOLATED FROM SICK ANIMALS

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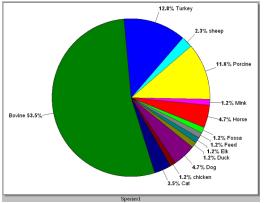
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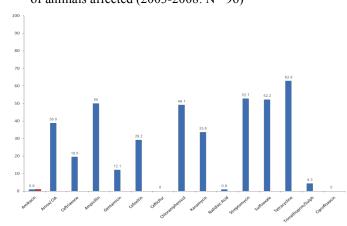
Salmonellosis is one of the most common foodborne diseases, and a major problem throughout the world (Zhao et al. 2003). Each year, thousands of human deaths occur as a result of salmonellosis. The disease is zoonotic and can affect animals too. Understanding the association between human salmonellosis cases and animal sources is an important epidemiological factor in the control and prevention of infection in humans. A previous study in North Dakota (ND) reported similarities in spatial and temporal trends in salmonellosis case reports in both domestic animals and humans (Oloya et al. 2007). Since the 1990's, strains of *Salmonella* have emerged that show antimicrobial resistance (AMR) which can lead to treatment failure in both animals and humans.

The objectives of this study were 1) to describe the distribution of salmonellosis cases in animals reported by the veterinary diagnostic laboratory at NDSU by time of submission, species of animals and county of origin and 2) to describe AMR patterns of *Salmonella* isolated and 3) determine associations between AMR and variables such as time of submission, species of animals and county of origin. The AMR testing was done using National Antimicrobial Resistance Monitoring System (NARMS) plates (Sensititre®, Trek Diagnostics System, Inc, Westlake, Ohio) against 15 antimicrobials. Data were entered in an excel spreadsheet and descriptive statistics run to describe the % resistance of antimicrobials tested, species of animals, time of diagnosis. EPI INFO version 3.2 used to map out distribution of salmonellosis cases by county and to run Chi square analysis to determine association between AMR and each variable.

The majority of species of animals affected were bovine (53.5%) followed by turkey (12.8%) and porcine (11.6%). A total of 22.1% of Salmonella occurred in other species that included horse & dog (4.7% each), cat (3.5%) sheep (2.3) and the rest (mink, fossa, feed, elk, duck and chicken) were 1.2% each (Figure 1). Most cases (31.4%) were reported in spring followed by winter (26.7%), fall (23.3%) and summer (18.6%). A map of North Dakota showing distribution of cases by county will be included in the presentation. AMR of the 15 antimicrobials tested ranged from 0.9% (Amikacin and Nalidixic Acid) to 62.8% (Tetracycline) Figure 2. All isolates tested (100%) were susceptible to Ciprofloaxin; however, drug resistance was found towards Tetracycline (62.8%), Streptomycin (52.7%), Sulfizoxale (52.2%), Ampicillin (50.0%), Chloramphenicol (49.1%), Amox/CIA (38.8%), Kanamycin (33.6%), Cefoxitin (29.2%), Ceftriaxone (19.5%), Gentamicin (12.1%), Trimethooprim/Sulph (4.3%), Amikacin (0.9%), and Nalidixic Acid (0.9%). Ceftiofur was non- interpretable. There was no significant association between AMR and season of diagnosis. These data show widespread AMR among *Salmonella* isolated from animals in ND.

Figure 1: Reports of salmonellosis cases ND by species Figure 2: Percent Antimicrobial Resistance to *Salmonella* of animals affected (2003-2008: N = 96)





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EFFECTS OF NUTRIENT RESTRICTION AND DIETARY SELENIUM ON EXPRESSION OF GAP JUNCTIONAL PROTEIN CONNEXIN (CX) 43 IN FETAL OVARIES OBTAINED FROM SHEEP IN LATE PREGNANCY; IMPLICATIONS FOR DEVELOPMENTAL PROGRAMMING.

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The fetal ovaries represent a type of tissue which grows rapidly. This process must be tightly regulated and requires cellular interactions which may be mediated through contact-independent and contact-dependent gap junctional communication. Gap junctions are composed of connexin proteins that form intercellular channels allowing for direct communication among cells, and are involved in growth control. During folliculogenesis in the fetus, primordial follicles develop to primary follicles, then secondary, and antral follicles. Maternal diet can affect fetal growth and organ function. Selenium (Se) is a mineral that has diverse biological functions. Selenium affects cellular proliferation in selected organs. We hypothesized that maternal diet will affect expression of Cx43 in fetal ovaries. Therefore, the aim of this study was to determine if maternal consumption of differing levels of energy and Se in diet impacts expression of Cx43 protein in fetal ovaries.

Sheep (n=26) were fed a maintenance (M; 2.12 Mcal/kg) or an energy restricted (R; 60% of maintenance) diet with high (H) Se (81.5 μg/kg body weight) or adequate (A) Se (7.4 μg/kg body weight) concentration from 21 days before breeding to day 135 of pregnancy. On day 135 of pregnancy fetal ovaries were collected and fixed. Ovaries (n=5-7/nutrition treatment) were sectioned and then immunostained for the presence of Cx43. To determine the Cx43 expression (percentage of positive staining out of the total follicle or tissue area), digital images of the tissues containing primary (total 33; 6-11/treatment), secondary (total 118; 19-36/treatment) and antral (total 153; 14-67/treatment) follicles were taken and analyzed using a computerized image analysis program. For primary and secondary follicles, Cx43 expression was determined in the granulosa layer; but for antral follicles, granulosa and thecal layers were analyzed separately.

Cx43 protein was expressed in fetal ovaries and was localized to primordial follicles, the granulosa layer of primary, secondary, and antral follicles, to theca layer of antral follicles, and an area between the oocyte and granulosa layer. Cx43 expression was greater (P<0.001) in granulosa cells of antral follicles than in primary or secondary follicles (1.5  $\pm$  0.1 vs. 0.5 $\pm$ 0.1 and 0.6  $\pm$  0.05%). Cx43 expression was greater (P<0.001) in granulosa than theca cells of antral follicles (1.5  $\pm$  0.1 vs. 0.6  $\pm$  0.1%). For primary and secondary follicles, maternal diet did not affect Cx43 expression. For granulosa and theca cells of antral follicles, Cx43 expression was greater (P<0.01) in ewes fed M diet with HSe than any other treatment groups.

These results demonstrate that 1) expression of Cx43 increases with follicular development from primordial to antral stage; 2) expression of Cx43 in granulosa layer is greater than in theca layer which is likely due to the avascular nature of the granulosa layer, and 3) both level of energy and Se in maternal diet affect Cx43 expression in antral follicles. These results emphasize the importance of maternal diet in fetal growth and development.

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#### BACTERIAL POPULATION STRUCTURE OF A GASIFICATION COOLING TOWER

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#### Introduction

The aqueous bacterial community composition of the Dakota Gasification Company's cooling tower in Beulah, ND was determined using Gram staining and phylogenetic analysis of extracted rRNA genes. Dakota Gasification is unique in the United States in its endeavor to gasify coal and convert it to synthetic natural gas, and relies on these bacterial populations for their ability to degrade the organic contaminates found in the wastewater produced in the process. On occasion, the cooling tower population densities crash, effectively limiting hydrocarbon removal and waste reduction in cooling tower waste treatment. The goal of this survey was to analyze the bacterial population composition in order to determine possible methods of crash prevention and recovery.

#### **Materials and Methods**

Nucleic acids were extracted as previously described (1) from Dakota Gasification's cooling tower water. Bacterial 16S rDNA was PCR amplified using bacterial specific primers 8F and 1391R under standard conditions and cloned into pCR 4.0 vectors per manufactures directions (Invitrogen, Carlsbad, CA). Phylogenetic analysis was done using Bayesian, maximum-likelihood and neighbor-joining algorithms.

#### **Results and Discussion**

The phylogenetic analysis of the extracted rRNA genes from 28 randomly selected bacteria revealed ten individual Gram-negative species from the β-proteobacteria and γ-proteobacteria phylum's (fig. 1). Approximately 68% of the cooling tower species tally shared 98% genetic identity with Comamonas denitrificans. Two of the targeted bacteria exhibited close relationships with Oligella urethralis (93% identity) and Pusillimonas noertemannii (94% identity). The remaining sample species exhibited close relationship to the genus Psuedomonas. Though phylogenetic analysis revealed significant species diversity,



Figure 1. Phylogenetic relationships of bacteria within the cooling tower environment. Sequences in bold represent those in identified in this study. All other sequences were retrieved from public databases.

there were three important characteristics shared throughout the populations. These shared traits were dissimilatory nitrate reduction (denitrification), the capacity to exploit a multitude of complex organic molecules as a source of carbon, and tolerance for elevated concentrations of heavy metals; all of which are characteristics shared with microbial communities found in activated sludge. These similarities allowed for the development of simple crash recovery procedures including strain purchase and reintroduction, species cultivation and repopulation, and on-site cultivation and storage for future use.

Our analysis also permits an estimate of coverage or what percentage of the bacterial community is represent by the 10 species we identified. We used Good's Coverage Estimator to estimate that these 10 species represent approximately 71.4% of the total number of bacterial cells in this community.

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#### EXAMINING THE ENVIRONMENTAL AND GENETIC CONTROL OF ESCHERICHIA COLI BIOFILM FORMATION

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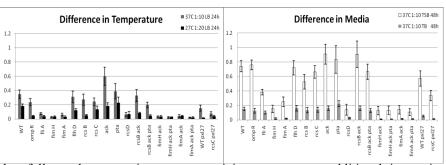
Accumulation of bacteria on various interfaces leads to the production of biofilms. Biofilm-associated bacteria are embedded in an extracellular matrix secreted by the bacteria themselves. According to Centers for Disease Control and Prevention, biofilms are estimated to be involved in about 65% of human bacterial infections (1). Biofilm formation is dependent on the environmental conditions, sensed by varies regulators. In this high-throughput study, we are focused on determining the environmental and genetic factors responsible for biofilm formation in *Escherichia coli*.

For this study, we took combinations of four environmental conditions (i.e. variable temperatures, medium, cell dilutions and incubation times) and nineteen strains of *E. coli* which includes the wild-type strain, AJW678, and a set of isogenic mutants in varies cell surface organelle or regulator genes. In order to reduce the number of experiments but at the same time cover as many conditions as possible, we used the D-Optimal response surface design algorithm (Design-Expert version 6.0.7, Stat-Ease Inc., Minneapolis MN and JMP 6, SAS Institute Inc., Cary NC).

The bacterial strains were first grown on Luria Bertani plates (LB; 1.5% agar, 1% tryptone, 0.5% NaCl, 0.5% yeast extract) and incubated overnight at 34°C. They were then grown in appropriate liquid media from which they were transferred to 96 well plates in appropriate dilutions. Plates were incubated without shaking at the designated temperature. In order to quantify the biofilms, an established crystal violet (CV) assay was used (2). Briefly, the biofilms were stained with 0.1% CV solution; the CV was solubilized with 80% ethanol/20% acetone. Optical densities were determined at 600 nm.

From the data collected we can say conclusively that out of the four environmental conditions, temperature and media cause the highest variation in biofilm formation among the strains (Fig. 1). At 37°C, all the *E. coli* strains show better biofilm formation compared to that at 27°C. In the nutrien rich tryptone soy broth (TSB), all strains produced more biofilm than in the nutrient poor tryptone broth (TB).

Fig. 1: Biofilm formation in wildtype and the mutants. The left panel compares two different temperatures, the right panel two different culture media.



Using a novel algorithm that follows the vector-item pattern mining concept, an additional interesting observation was made. We related functional annotations (Gene Ontology, GO) of the proteins that are encoded by the mutated genes to quantitative amounts and found that the GO that relates to 'pyruvate catabolic process' was significant if GOs that related to the type I fimbrium were omitted. Mutants in any of the components of the type I fimbrium did not produce any biofilm under any of the conditions tested. Apparently, attachment is crucial to bioiflm formation. Mutants relating to pyruvate catabolism were in acetate kinase (Ack) and phosphotransacetylase (Pta) which are part of acetate metabolism. We conclude that acetate or one of its intermediates are benefitial to biofilm formation. Another mutant that was consistently low in biofilm production was the *rcsD* mutant that affects the production of the capsule

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The authors thank Shane Stafslien and David Christianson (Center for Nanoscale Science and Engineering, NDSU, Fargo ND) for their help with selecting the environmental conditions that were used for this study. The work was funded by the ND Agricultural Experiment Station

### THE 5TM VPAC1 ISOFORM BLOCKS CAMP SIGNALING EVOKED FROM THE FULL-LENGTH 7TM RECEPTOR

Erich Raymond Wilkerson<sub>1,2</sub>, Rebecca J Hermann<sub>1,2</sub>, Donald R Branch<sub>3</sub>, Glenn Dorsam<sub>1,2</sub>.

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Vasoactive intestinal peptide receptor 1 (VPAC1) couples heterotrimeric G proteins, Gαs and Gαi, that activate and inhibit adenylate cyclase cAMP production. The 7 transmembrane (TM) VPAC1 isoform, increases intracellularcAMP ([cAMP]i) upon binding its ligand, vasoactive intestinalpeptide (VIP). In contrast, the 5TM VPAC1 isoform fails toincrease [cAMP]i over a range of 10-6-10-12 M VIP. Our hypothesis is that the dual expression of both the 5 and 7TMisoforms cause a lack of [cAMP]i elevation due to an antagonisteffect by the 5TM on 7TM signaling. To investigate this, human HuT 78 cells, a malignant T cell, which express both 5 and 7TMVPAC1 isoforms. Levels of [cAMP]i were measured by acompetitive ELISA after treating with increasing concentrations of VIP. Interesting, we show a VIP dependent decrease in [cAMP]i, suggesting that the 5TM VPAC1 isoform coexpressed in HuT 78 cells suppress 7 TM induced [cAMP]i levels. The ratio of the 5 and 7TM receptors on the cell membrane may explain the unique level of [cAMP]i upon VIP binding. Future studies will inhibit Gαi coupling with pertussis toxin and RNAi to knockdown the 5TM VPAC1 to measure [cAMP]i response evoked by the 7TM VPAC-1 receptor in coexpressing cells.

Research supported by NIH-KO1 1KO1DK664828 and COBRE 2P20RR015566.

### VASCULAR GROWTH IN UTERINE TISSUES DURING EARLY PREGNANCY IN SHEEP

Robert Wroblewski, Pawel P Borowicz, Dale A Redmer, Lawrence P Reynolds and Anna T Grazul-Bilska.

#### Center for Nutrition and Pregnancy, and Department of Animal Sciences, North Dakota State University, Fargo.

Placental vascular development (angiogenesis) is critical for normal placental function and thus for normal embryonic/fetal growth and development. Numerous factors, including environmental factors or application of assisted reproductive techniques (ART) affect embryonic development. In fact, we and others have shown that embryos from ART frequently exhibit decreased cell proliferation and/or poor placental angiogenesis, which may contribute to their high rate of loss after transfer (1, 2, 3). We hypothesized that vascular growth will change during early pregnancy. Therefore, the objective of this study was to determine cellular proliferation within blood vessels, and several measurements of capillary/blood vessel growth in uterine tissues from day 14 to day 30 of pregnancy.

Uterine tissues were collected on days 14, 16, 18, 20, 22, 24, 26, 28, and 30 after mating (n = 5-6/day) and on day 9-11 after estrus (n = 5; non-pregnant control). To maintain the morphology, specimen-pins were inserted completely through the uterus and fetal membranes at the level of the external intercornual bifurcation, cross-sections (0.5-cm thick) were taken, and tissues were immersion-fixed in Carnoy's solution and embedded in paraffin. Tissue sections were stained with periodic acid-Schiff's reagent and immunostained to detect proliferating cell nuclear antigen (PCNA, a marker of proliferating cells), followed by image analysis of uterine caruncular areas.

Compared with nonpregnant controls, vascular labeling index (proportion of proliferating cells within blood vessels) increased (P < 0.001) ~2-fold on days 14-18, and 4- to 6-fold on days 20-30; whereas the tissue area occupied by capillaries increased (P < 0.03) by ~2-fold on days 22-30, and area per capillary increased 2- to 3-fold on days 22-30. However, number of capillaries per tissue area and capillary exchange surface did not change throughout early pregnancy.

These data indicate that uterine angiogenesis, manifested by increased vascular cell proliferation and subsequent enlargement of capillaries, is initiated very early in pregnancy, around the time of pregnancy recognition. These data provide a basis for determining whether placental vascular development is altered in compromised pregnancies. Understanding the causes of inadequate embryonic development in compromised pregnancies may help to establish strategies to rescue such pregnancies. Supported by NIH grant HL64141 to LPR and DAR, USDA grant 2007-01215 to LPR and ATGB, ND EPSCoR AURA grant to MAM and ATGB, and P20 RR016741 from the INBRE program of the National Center for Research Resource.

- 1) Reynolds LP, Redmer DA. (1995) Utero-placental vascular development and placental function. J. Anim. Sci. 73:1839-1851.
- 2) Reynolds LP, Borowicz PP, Vonnahme KA, Johnson ML, Grazul-Bilska AT, Redmer DA, Caton JS. (2005) Placental angiogenesis in sheep models of compromised pregnancy. J. Physiol. 565:43-58.
- 3) Reynolds LP, Caton JS, Redmer DA, Grazul-Bilska AT, Vonnahme KA, Borowicz PP, Luther JS, Wallace JM, Wu G, and Spence TE. (2006) Evidence for altered placental blood flow and vascularity in compromised pregnancies. J. Physiol., 572: 51-58.

#### RAPID SYNTHESIS OF PIPERONYLFORMAMIDE

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Piperonylamine is an important intermediate in the synthesis of biologically active compounds, including agrochemicals and pharmaceuticals. Piperonylamine can be synthesized from piperonal by the Leuckart reaction via the intermediate piperonylformamide. In the literature, the synthesis of piperonylformamide was described only once, and it appeared to be a very slow reaction, taking 11 hours to complete. Recently, we developed an accelerated procedure for the synthesis of formamide fungicides via the Leuckart reaction. In this work, the accelerated procedure was successfully applied to the synthesis of piperonylformamide from piperonal. The reaction was fully completed in 1 minute and produced piperonylformamide in good yield. The new reaction opens the way for the fast synthesis of piperonylamine and its derivatives in the laboratory practice and industry.

The project is supported by NIH grant P20 RR016741 from the NCRR.

### OPTIMIZATION OF GOLD NANOPARTICLE SIZE FOR USE IN LATERAL FLOW BIOSENSORS

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Gold nanoparticles have attracted considerable interest for biosensor and bioassay due to their unique optical properties. In this study, gold nanoparticles with various sizes were used as labels to develop lateral flow test strip biosensors for the detection of IgM. The total assay time was 15 min and the resulting signals were quantitatively measured using a hand-held strip reader. It was found that 40 nm gold nanoparticles provided the optimal signal for this detection. This method provided a detection range up to 200 ng/mL with a detection limit of 0.25 ng/mL. The ability of the biosensor to detect carcinoembryonic antigen (CEA) using the 40 nm gold nanoparticles was also studied. The use of these nanoparticles along with the optimization of parameters resulted in a reliable method for the quantitative detection of this protein biomarker.

### ANALYSIS OF CHROMATIN STRUCTURE IN THE MYELOID-LYMPHOID LEUKEMIA GENE TRANSLOCATION BREAKPOINT CLUSTER REGION

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Department of Biology, Minot State University, Minot, North Dakota 58707

The Myeloid-Lymphoid Leukemia (MLL) gene fuses to >50 different loci as the result of reciprocal translocations associated with several subtypes of human acute leukemia. In every case, the fusion point in MLL is within the same 8 kilobase pair (kbp) region, called the MLL breakpoint cluster region (bcr). Despite this conserved breakpoint, no translocation mechanism has been definitively described. Recently, electrophoretic mobility shift assay (EMSA) was used to determine protein binding characteristics in the MLL bcr. Proteins were noted to bind at the extreme boundaries of the MLL bcr, but not in an internal region noted to be a hot spot for breakage in leukemia-associated translocations. The limited mobility observed with the protein-DNA complexes indicate a large protein or multiple proteins may bind at the 5' and 3' ends of the MLL bcr. Binding occurred with nuclear proteins from hematopoetic cells, but not with nuclear proteins from fibroblast cells and was sequence-specific as shown by competitor assays. Analysis of the most 5' region of the MLL ber showed several discontinuous regions are important for protein binding. Our results suggest that specific protein-DNA interactions may define the limits of the MLL bcr. Binding of proteins at the boundaries of the MLL bcr prompted a study of chromatin analysis within the regions. Chromatin immunoprecipitation assay (ChIP) showed a histone H3-free region at the extreme 5' end of the MLL bcr suggesting this is a nucleosome-depleted region. The 3' boundary showed slightly more H3 binding than the 5' end but considerably less than a control nucleosome-occupied region. The internal breakage hot spot showed prominent H3 binding consistent with the presence of nucleosomes. The chromatin study suggests that the MLL ber boundary regions are more exposed, especially in the 5' region. This further supports our EMSA findings, that the MLL bcr is likely bordered by DNA-protein complexes. Future studies will include additional chromatin analysis including DNase I hypersensitivity in an effort to further describe chromatin structure in the MLL bcr. The project described was supported by NIH Grant Number P20 RR016741 from the North Dakota INBRE Program of the National Center for Research Resource, and by a Minot State University institutional research grant.

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GRADUATE COMMUNICATIONS

IN THE

A ROGER DENISON COMPETITION

#### SCHEDULE OF PRESENTATIONS - 'GRADUATE SESSION #1

Graduate session #1 talks will be in the Arikara room in the Memorial Union – session will be chaired by A. Rodger Denison Competition judges

#### MORNING SESSION

- 7:30 Registration desk in the Legacy Lounge in the Memorial Union open
- 8:00 Greetings from President Prüß in the Century Theatre in the Memorial Union
- 8:20 THE TUMOR SUPPRESSOR IKAROS ENGAGES THE VPAC1 PROMOTER IN ACTIVATED HUT 78 CELLS. Keith Benton, Rebecca Hermann, Sheri Dorsam, Travis Van der Steen, Ashley Nelson, Sinisa Dovat and Glenn Dorsam. [p. 41]
- 8:40 A PROTEOMIC INVESTIGATION OF *ESCHERICHIA COLI* BIOFILM FORMATION ON A SILICONE COATING CONTAINING A TETHERED QUATERNARY AMMONIUM COMPOUND. Justin W. Daniels, Shane J. Stafslien, Bret J. Chisholm, Steven Meinhardt, Birgit M. Prüß [p.43]
- 9:00 ISOLATION OF POLYKETIDE SYNTHASE GENES ASSOCIATED WITH SOLANAPYRONE PRODUCTION IN *ASCOCHYTA RABIEI*. Javier A. Delgado, Steven Meinhardt, Sam G. Markell and Rubella S. Goswami. [p.44]
- 9:20 Aβo STIMULATES MICROGLIA THROUGH A TYROSINE KINASE ACTIVATION. Gunjan Dhawan, Colin.K.Combs . [p.45]
- 9:40 FUSARIUM SPECIES ASSOCIATED WITH ROOT ROT OF DRY BEANS IN NORTH DAKOTA. Aakansha Gambhir, Robin S. Lamppa, Jack B. Rasmussen and Rubella S. Goswami. [p.46]
- 10:00 PROTEOMIC PROFILING AND ANTIGENIC CHARACTERIZATION OF EXTENSIVELY-DRUG RESISTANT *ESCHERICHIA COLI* STRAINS ISOLATED FROM SCOURING CALVES. Ablesh Gautam\*, Heather Vinson, <sup>1</sup>Penelope S. Gibbs, and Robert Barigye [p.47]
- 10:20 BREAK (refreshments and exhibits in the Plains Room in the Memorial Union)
- 10:40 ALPHA-1A ADRENERGIC RECEPTOR ACTIVATION ENHANCES
  NEUROGENESIS AND COGNITIVE FUNCTION. Brianna Goldenstein, Brian
  Nelson, Sarah Boese, Danielle Schlosser, Chris Knudson, Patrick Carr, Dianne Perez,
  Van Doze [p.48]
- 11:00 THE ROLE OF B1 AND B2 CELLS IN ALLERGIC ASTHMA. Sumit Ghosh, Scott A. Hoselton and Jane M. Schuh. [p.49]
- 11:20 EFFECTS OF MATERNAL NUTRITION AND SELENIUM STATUS ON OFFSPRING CUTOUT.. E. Harris, J. Caton, P. Berg, K. Vonnahme, D. Redmer, L. Reynolds, and K. Maddock Carlin. [p.50]
- 11:40 THE FIVE TRANSMEMBRANE VASOACTIVE INTESTINAL PEPTIDE RECEPTOR 1

ISOFORM IS EXPRESSED IN HUMAN BLOOD CD14+ MONOCYTE CELLS. Rebecca J Hermann, Travis Van der Steen, Jarrett Failing, Donald R Branch, Glenn Dorsam. [p.51]

12:00 LUNCH (served in the Plains Room in the Memorial Union). We will also conduct our business meeting (open to all members) during the lunch hour.

#### AFTERNOON SESSION

- 1:00 α<sub>1A</sub> ADRENERGIC RECEPTOR ENHANCEMENT OF NEUROGENESIS AND INTERNEURON FUNCTION. Chris WD Jurgens, Dianne M Perez, Van A Doze [p.53]
- 1:20 FUNGAL VIABILITY INFLUENCES THE IMMUNE RESPONSE IN ALLERGIC ASTHMA. Sumali Kapoor, Scott Hoselton, and Jane Schuh. [p. 54]
- 1:40 EFFECT OF MATERNAL DIET ON FETAL:MATERNAL RATIO OF CIRCULATING AMINO ACIDS, NON-ESTERIFIED FATTY ACIDS, BLOOD UREA NITROGEN, AND GLUCOSE CONCENTRATIONS IN EWES. L. A. Lekatz, G. Wu, L. P. Reynolds, D. A. Redmer, J. S. Caton, and K. A. Vonnahme. [p.56]
- 2:00 CHARACTERISATION OF ANTIMICROBIAL RESISTANCE (AMR) AND PRESENCE OF CLASS 1 INTEGRONS IN *SALMONELLA* SEROVARS ISOLATED FROM CLINICAL CASES OF ANIMALS AND HUMANS IN NORTH DAKOTA. Michael Mahero, Susan Olet, Doekott Dawn, Margaret L Khaitsa. [p.58]
- 2:20 RGS7 PROTEIN SUPPRESSION OF  $G\alpha_o$  Protein-Mediated  $\alpha_{2A}$ -ADRENERGIC RECEPTOR INHIBITION OF MOUSE HIPPOCAMPAL CA3 EPILEPTIFORM ACTIVITY. Brian Nelson, Brianna Goldenstein, Ke Xu, Elizabeth Luger, Jacquline Pribula, Jenna Wald, Lorraine O'Shea, David Weinshenker, Raelene Charbeneau, Xinyan Huang, Richard Neubig, Van Doze. [p.61]
- 2:40 BREAK (served in the Plains Room in the Memorial Union)
- 3:00 ANTIBODY PRODUCTION IS PROMOTED IN THE ABSENCE OF VPAC-2 RECEPTOR IN A MURINE MODEL OF ALLERGIC ASTHMA. Amali Samarasinghe\*, Scott Hoselton, and Jane Schuh. [p.62]
- 3:20 CHARACTERIZATION OF THE 2007 OUTBREAK OF NEPHROTOXICITY AMONG DOGS AND CATS ASSOCIATED WITH MELAMINE IN PET FOOD. Stella Opendi Sasanya, Susan Olet, Robert Littlefield and Margaret L. Khaitsa. [p.63]
- 3:40 POSTTRANSLATIONAL REGULATION OF THE TUMOR-SUPPRESSOR IKAROS BY VASOACTIVE INTESTINAL PEPTIDE RECEPTOR 1 IN HUMAN HUT 78 CELLS Travis Van der Steen, Steven Meinhardt, Sinisa Dovat and Glenn Dorsam. [p.65]
- 4:00 *IN VIVO* OPPOSING REGULATION OF VASOACTIVE INTESTINAL PEPTIDE RECEPTOR-1 AND -2 THROUGHOUT THE T CELL IMMUNE RESPONSE. Emilie Vomhof-DeKrey, Jodie Haring, Glenn Dorsam. [p.66]
- 4:20 CAVEOLAR MICRODOMAINS AS ORGANIZERS OF CALCIUM SIGNALING.

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Biswaranjan Pani, Hweiling Ong\*, Xibao Liu\*, Kristina Rauser, Virginia Achen, Indu Ambudkar\* and Brij B Singh [p.68]

4:40 INCREASING THE SENSITIVITY OF ADVANCED STAGE OF PROSTATE CANCER TO CHEMOTHERAPEUTIC DRUGS-INDUCED APOPTOSIS BY TARGETING *miR-205* AND *miR-31*. Namrata Bhatnagar, Xia Li and Bin Guo [p.69]

#### **EVENING**

6:00 Banquet will be in the Plains Room in the Memorial Union)

GRADUATE SESSION #2 CONTINUED ON NEXT PAGE.

### SCHEDULE OF PRESENTATIONS GRADUATE SESSION #2

Graduate session #2 talks will be in the Peace Garden Room in the Memorial Union – session will be chaired by A. Rodger Denison Competition judges

- 7:30 Registration desk in the Legacy Lounge in the Memorial Union open
- 8:00 Greetings from President Prüß in the Century Theatre in the Memorial Union
- 12:00 Lunch (served in the Plains Room in the Memorial Union). We will also conduct our business meeting (open to all members) during the lunch hour

#### AFTERNOON SESSION

- 1:00 VARIATIONS IN THE LAST DAY OF SPRING FROST, THE FIRST DAY OF FALL FROST AND THE GROWING SEASON LENGTH IN NORTH DAKOTA. Ambika Badh<sup>1\*</sup>, Adnan Akyuz, Gary Vocke and Barbara Mullins. [p.39]
- 1:20 DISPOSABLE NUCLEIC ACID BIOSENSORS BASED ON GOLD NANOPARTICLE PROBES AND LATERAL FLOW STRIP. Meenu Baloda, Xun Mao, and Guodong Liu [p.40]
- 1:40 CHANGES IN MOLLUSCAN SPECIES ACROSS THE K/PG BOUNDARY; SELECTED SITES IN MONTANA, NORTH DAKOTA, AND ALBERTA. Anna M. Crowell\* and Joseph H. Hartman. [p.42]
- 2:00 DETECTION OF DNA SEQUENCES USING GOLD NANOROD ENHANCED FLUOROPHORES. Carrie L. John, \*Shaina L. Strating and Julia Xiaojun Zhao. [p.52]
- 2:20 POSSIBLE EFFECT OF CARBON NANOTUBE CRYSTAL STRUCTURE ON GAS-SURFACE INTERACTIONS-THE CASE OF BENZENE, WATER AND N-PENTANE ADSORPTION ON SWCNTS. M. Komarneni,\*A. Sand, J. Goering, and U. Burghaus. [p.55]
- 2:40 BREAK (refreshments and exhibits in Plains Room in Memorial Union).
- 3:00 *IN SITU* GROWTH OF AU NANORODS ON TIO<sub>2</sub> SURFACES FOR PHOTOELECTROCHEMICAL SOLAR CELLS. Aize Li, Nenny Fahruddin, David T. Pierce, and Julia X. Zhao. [p.57]
- 3:20 MULTIPLEX ELECTROCHEMICAL IMMUNOASSAY USING GOLD NANOPARTICLE PROBES AND IMMUNOCHROMATOGRAPHIC STRIPS. Xun Mao, Meenu Baloda, Anant S. Gurung, Yuehe Lin, Guodong Liu. [p.59]
- 3:40 ROUGH CALCULATIONS PERTAINING TO SCANNING TUNNELING MICROSCOPY AND ANGLE RESOLVED PHOTOEMISSION SPECTROSCOPY ON GRAPHENE M. L. C. Murdock and W. Schwalm. [p.60]
- 4:00 THE PALEONTOLOGY AND BIOSTRATIGRAPHY OF THE TURTLE BUTTE FORMATION, TRIPP COUNTY, SOUTH DAKOTA. Karew Schumaker [p.64]
- 4:20 A NEW INTERPRETATION OF THE PALEOECOLOGY OF THE BIG PIG DIG QUARRY,

PROCEEDINGS OF THE NORTH DAKOTA ACADEMY OF SCIENCE, VOL. 62, 2008

## BADLANDS NATIONAL PARK, SOUTH DAKOTA. Matthew W. Weiler [p.67]

## **EVENING**

6:00 Banquet will be in the Plains Room in the Memorial Union

# VARIATIONS IN THE LAST DAY OF SPRING FROST, THE FIRST DAY OF FALL FROST AND THE GROWING SEASON LENGTH IN NORTH DAKOTA

Ambika Badh<sup>1\*</sup>, Adnan Akyuz<sup>1</sup>, Gary Vocke<sup>2</sup> and Barbara Mullins<sup>1</sup>

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Growing season length for eight stations of North Dakota, Northern United States from 1879 to 2008, as well as the first and the last day of frost (based on air temperature being 0°C or lower) was tabulated and analyzed. Fargo, Bismarck, Jamestown, Williston, Minot, Pembina, Dickinson and Langdon were selected based on the accuracy of data, length of period, and availability with the least missing data since the historical climate records have been recorded for those stations. They also provided a diverse spatial resolution to cover the eight most prime agricultural locations of the state. Growing season for this region was defined as the period between the first and the last day of frost. The growing season length for the state on an average showed to lengthen by 1.1 days per decade. On analyzing the data further, it was found that the fluctuations in the last day of spring frost since 1879 for all the eight stations was more prominent than the fluctuations in the first day of fall frost.

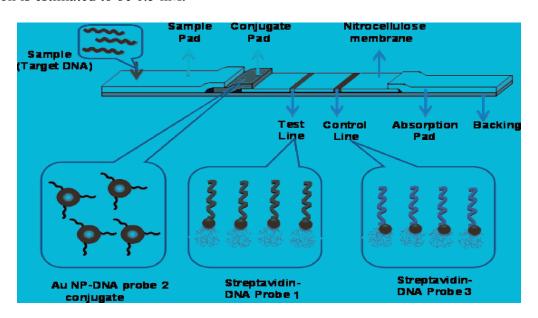
The objective of this study was to study the relationship between these fluctuations and the growing season length for the state. Trends showing the fluctuation in the first day of fall frost and the last day of the spring frost from 1879 were tabulated for all of the eight stations. It was concluded from this study that the greater uncertainty related to the occurrences of the last day of spring frost was more accountable for the changes in the trend of growing season length.

# DISPOSABLE NUCLEIC ACID BIOSENSORS BASED ON GOLD NANOPARTICLE PROBES AND LATERAL FLOW STRIP

### Meenu Baloda, Xun Mao, and Guodong Liu\*

## Department of Chemistry and Molecular Biology, North Dakota State University, Fargo, North Dakota 58105

Various strategies and technologies have been developed to identify unique DNA sequences. But the procedures used for identification of nucleic acids are laborious and time consuming. Therefore we described a Disposable Nucleic Acid Biosensor (DNAB) for low cost, sensitive and fast detection of nucleic acid samples. (1) The DNA hybridization reaction was carried out on the lateral flow chromatographic strips with help of gold nanoparticles. The gold nanoparticle probe was captured on the test zone which produced a red line. This visual detection can be done in 15 minutes. The quantitative measurement of the signal was recorded through a portable strip reader. Experimental parameters including the amount of DNA probe in the test zone, amount of gold-Nanoparticle conjugate on the conjugate pad, the components of the running buffer as well as the pretreatment conditions of the sample pad were optimized systematically. The response of the optimized device is highly linear over the range of 1-100nM target DNA, and the limit of detection is estimated to be 0.5 nM.



Schematic illustration of the DNAB

We conclude that a DNAB was successfully developed for fast, easy, low cost sensitive detection of nucleic acid samples which shows great promise for in-field and point-of- care diagnostics of genetic diseases and infectious agents.

(1) Mao, X, Ma, Y, Zhang, A, Zhang L, Zeng, L and Liu, G *Anal. Chem.*, **2009**, *81*(4), pp 1660–1668

# THE TUMOR SUPPRESSOR IKAROS ENGAGES THE VPAC1 PROMOTER IN ACTIVATED HUT 78 CELLS

Keith Benton<sup>1</sup>, Rebecca Hermann<sup>1</sup>, Sheri Dorsam<sup>1</sup>, Travis Van der Steen<sup>1</sup>, Ashley Nelson<sup>1</sup>, Sinisa Dovat<sup>2</sup> and Glenn Dorsam<sup>1</sup>.

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DNA sequence specific recruitment of transcription factors to gene promoters is essential for normal gene expression. Total Ikaros (IK) protein and its phosphorylation pattern change during activation of lymphocytes, altering its subnuclear localization and DNA binding. IK has been shown to decrease expression of an anti-proliferative, G-protein coupled receptor, vasoactive intestinal peptide receptor 1 (VPAC1) when ectopically overexpressed in NIH-3T3 cells. The VPAC1 promoter contains 24 putative binding sites for IK, including four high affinity motifs. VPAC1 mRNA levels are dependent on the activation status of CD4 T cells, as TCR signaling downregulates VPAC1 expression. IK is hypothesized to engage the VPAC1 promoter upon activation and downregulate expression. Binding of IK to the VPAC1 promoter was queried using chromatin immunoprecipitation (ChIP). IK was enriched at the VPAC1 promoter in an activation (PMA/ionomycin) dependent manner in HuT 78 cells, but surprisingly, did not result in a decrease of VPAC1 expression. Future studies will overexpress DNA binding, non DNA binding, and alanine/aspartate mutant IK isoforms to determine their effect on IK binding. Engagement of the master regulator of lymphopoesis, IK, at the neuropeptide receptor VPAC1 promoter strengthens the neuroimmunomodulation connection at a molecular level.

## CHANGES IN MOLLUSCAN SPECIES ACROSS THE K/PG BOUNDARY; SELECTED SITES IN MONTANA, NORTH DAKOTA, AND ALBERTA

### Anna M. Crowell\* and Joseph H. Hartman

### Department of Geology and Geological Engineering, University of North Dakota, Grand Forks, ND

HYPOTHESIS. Previous sampling methods of continental mollusks created a sampling bias because smaller species (specimens) likely present in faunules were overlooked. Thus, an oversimplified view may have been obtained concerning changes in population structure (local faunas), species diversity, and paleoenvironment across the Cretaceous–Paleogene (K/Pg) boundary or during other times through geologic time. Specific awareness of sampling for smaller-sized species can also lead to a better overall view of the in situ environmental conditions (autochthonous) of end Cretaceous and initial Paleogene times. Also, study of microto other under-sampled mollusks permits a better interpretation of hypotheses regarding species diversity and morphological disparity before and after the K/Pg extinction event. Quitting Time (L6771a-I), a Montana locality, is of particular interest because of the large number of sphaeriids that coexisted with gastropods and other taxa, gastropods being the primary microrecord at the other seven localities under study.

**INTRODUCTION**. Using the iridium anomaly, the presence of palynomorphs, and relative stratigraphic position (1), eight localities were selected for the purpose of this study, four above and below the boundary. The stratigraphic order of occurrences is Hell Creek Formation (Montana, Locality L6771, -8.9 m; North Dakota, Locality, L6521, -2.71 m); Willow Creek Formation (Alberta, L6717a, -1.41 m; L6717b, -0.56 m; L6717c, +0.08 m); Tullock Member, Fort Union Formation (Montana, Locality L5241, +1.7 m; L6594, ~+4.1 m); and Bear Member, Fort Union Formation (Montana, Locality L6107a, b, ~+8.7 m).

**METHODS**. As noted, the local faunas under study were collected from eight geographically separated locations spaced above and four below the K/Pg boundary. Specimens were separated from their fine-grained matrix by hand and identified with the aid of photo- and scanning electron microscope imaging. Species relative abundance at each locality and taphonomic (fabric) conditions in matrix were recorded in a spreadsheet, where the data were manipulated to examine population patterns and locality species variability.

**RESULTS**. Apart from sphaeriids, *Campeloma* and *Lioplacodes* species are particularly abundant at the Cretaceous localities, with *Succinea* and *Aplexa* not occurring at the Paleocene study localities. Species of *Lioplacodes* and cf. *Gyraulus* are abundantly represented in the Paleocene localities, with rare conchostracans, *Pleurolimnaea*, and planorbids. *Campeloma* are also present at the Paleocene localities, although in lesser abundance than at Cretaceous localities. Other species located above and below the boundary include *Physa*, "*Hydrobia*," *Acroloxus*?, New Genus A *limneaformis*, opercula specimens, and ostracods. Quitting Time has abundant examples of *Sphaerium beckmani*, *S. cf. S. subellipticum* and many types of mussels, including *Plesielliptio whitmani* and *Plethobasus*.

**DISCUSSION**. Campeloma (n. sp.), Lioplacodes (including L. tenuicarinata), and cf. Gyraulus survived the extinction event without question. Others, such as Succinea and Pleurolimnaea, are only represented before or after, respectively. Species from slow-moving water environments appear to have, for the most part, survived the extinction event quite well, as massive loss of microtaxa is not apparent with this data set, in contrast to the larger freshwater mussels (2).

<sup>1)</sup> Crowell A, Hartman JH, and Sweet AR (2008) Geol. Soc. Amer. Abs. with Programs., 40(6), 144.

<sup>2)</sup> Hartman JH (1998) in Johnston P, Haggart J, eds., Bivalves: An Eon of Evolution: Calgary, University of Calgary Press, 317–345.

# A PROTEOMIC INVESTIGATION OF ESCHERICHIA COLI BIOFILM FORMATION ON A SILICONE COATING CONTAINING A TETHERED QUATERNARY AMMONIUM COMPOUND

Justin W. Daniels\*1, Shane J. Stafslien1, Bret J. Chisholm2, Steven Meinhardt3, Birgit M. Prüß1

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Biofilms are communities of attached microorganisms that are embedded in an extracellular matrix that they themselves produce. These communities are known to be inherently resistant to a wide variety of antibiotics and disinfectants and are extremely difficult to eradicate once they are established on surfaces, such as implanted medical devices and food processing equipment. In the present study, we are investigating the differential protein expression during *Escherichia coli* biofilm formation on an unmodified silicone coating and a silicone coating containing a covalently tethered quaternary ammonium compound (QAC). Tethered QACs have been shown to exhibit highly effective, broad spectrum antimicrobial activity through a contactactive mechanism (1). It is anticipated that silicone coatings containing tethered QACs can be utilized to provide long term antimicrobial protection to surfaces that are susceptible to microbial biofilm formation.

*E.coli* biofilms were cultured in Tryptic Soy Broth on surfaces of the coatings prepared in multi-well plates (2). After incubation, the biofilms were rinsed to remove non-attached cells and analyzed with a suite of quantitative spectrophotometric techniques, including crystal violet (CV), alcian blue (AB) and ATP bioluminescence (LUM) assays. CV and AB dyes are used as total biomass indicators, staining anionic biomacromolecules and extracellular matrix carbohydrates, respectively. The ATP bioluminescence assay is used to measure cellular viability by reacting cellular ATP with a luciferase to produce luminescence. The results (Figure 1) showed a significant reduction in biofilm growth (as characterized by CV and AB) with no discernable reduction in bioluminescence, indicating that the production of biofilm extracellular matrix is being inhibited without affecting cellular viability on the surface of the QAC-containing silicone coating.

For proteomic analysis, the *E.coli* biofilms were recovered from the coating surfaces using nylon flocked swabs and recovered into 2mL of sterile deionized water. The recovered biofilm solutions were sonicated for six 10 second pulses, each at different power settings: 20%, 40%, 60% and 80%. The sonicated solutions were centrifuged to pellet cell debris and the supernatant was collected and concentrated for protein analysis. All samples were resolved in 10% and 12.5% Laemmli-SDS-PAGE gels to visualize band patterns. Gels were post stained with Deep Purple and images were obtained by scanning with the Ettan DIGE Imager. Based on the observed band patterns and the relative intensity of detected bands, it was determined that the 20% power setting yielded optimal protein extraction for lower molecular weight proteins (<25kD) while the 60% power

vielded optimal protein setting extraction for higher molecular weight proteins. This data suggests that multiple sonication settings need to be used for detection of differentially expressed proteins on these surfaces based on the MW range of interest. Optimization of 2D SDS-PAGE gels is currently being performed, which will be followed by mass spectrometry identification of the observed differentially expressed protein spots.

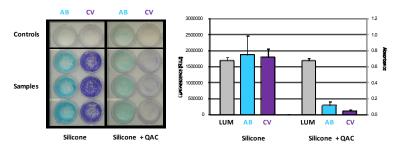


Figure 1. E.coli biofilm quantitative assay results

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## ISOLATION OF POLYKETIDE SYNTHASE GENES ASSOCIATED WITH SOLANAPYRONE PRODUCTION IN ASCOCHYTA RABIEI

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Ascochyta blight is the most important disease of chickpeas in North Dakota, causing severe yield losses and significantly reducing seed quality. It is caused by the necrotrophic fungal pathogen Ascochyta rabiei (Pass.) Labr and is largely spread through seeds. Ascochyta blight symptoms develop under cool, wet weather producing lesions in all above ground plant structures. The symptoms are visualized as concentric rings of black pycnidia within round brown to gray lesions. The infection process begins by the spore landing, germination, and adhesion on plant tissue, followed the development of appressoria (2). Production of cell wall degrading enzymes facilitates penetration of the cuticle (2, 5). The tissue penetration is also aided by mycotoxins secreted during sporulation (1), among which solanapyrones are the most studied in Ascochyta species (2, 3). They are secondary metabolites that have been correlated with seedling inhibition and crude extracts from fungal cultures have been shown to produce blight-like symptoms on chickpeas (1, 3). Solanapyrones have been reported as virulence factors in several Ascochyta species and in the potato and tomato pathogen Alternaria solani. They are known to be produced via a reduced-type polyketide synthase (RD-PKS) pathway. Some preliminary information about proteins involved in the synthesis of solanapyrones is available. However, genes involved in their production have not been cloned from Ascochyta rabiei. Solanapyrones are highly reactive and very difficult to detect in vivo. In fact, out of the various forms of solanapyrones, only one form has been detected in plant tissue (4). The objective of the study is to clone and characterize one or more key polyketide synthase (PKS) genes associated with the production of solanapyrones in Ascochyta rabiei, to establish their role in the solanapyrone biosynthetic pathway and their effect on pathogenicity of the fungus. Since no nucleotide sequence information was available for this fungus, cloning was initiated by aligning seven RD-PKS protein sequences available in Genbank, selecting conserved regions and designing degenerate primers. PCR fragments generated using the degenerate primers were cloned into pCR®4-TOPO vector, transformed into E. coli and sequenced. The translated consensus sequence obtained after aligning these clones matched a beta-ketoacyl synthase (KS) domain using the Conserved Domain Search (CDS) at NCBI website. The length of this sequence was increased further using a combination of specific and degenerate primers. The 1083 bp sequence obtained as a result of this effort was found to be similar to an RD-PKS involved in secondary metabolite production from the necrotrophic fungus Botryotinia fuckeliana, causal agent of gray mold, when compared using BLASTX. The region appeared to code for a KS domain according to CDS. This sequence is being currently being increased further using the genome walking strategy and is being used as a probe to screen an A. rabiei genome phage library. Progress towards cloning the polyketide synthase genes from A. rabiei and an evaluation of the suitability of the above mentioned techniques for fungal gene isolation will be presented.

Key words: Ascochyta blight, chickpea, polyketide synthase, solanapyrone

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## ABO STIMULATES MICROGLIA THROUGH A TYROSINE KINASE ACTIVATION

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Accumulation of beta amyloid (Aβ) peptide has been hypothesized to play a major role in pathogenesis of Alzheimer's disease. Aß fibrils are known to accumulate in diseased brains and exert neurotoxic effects both in vitro and in vivo. However, recent reports indicate the prefibrillar, oligomeric forms of the peptide may also be involved in mediating disease progression particularly in earlier stages of disease. Prior work from our laboratory as well as others suggests that soluble, oligomeric AB directly stimulates microgliosis and neuronal death/dysfunction in vitro. Using primary murine microglia cultures, this study continued to define the mechanism by which AB oligomers stimulate microglia to acquire a reactive phenotype. Aß oligomers versus fibrils stimulated microglia to increase secretion of the cytokine, tumor necrosis factor alpha. Microglial pre-treatment with the mixed Src family/Abl inhibitor, dasatinib, attenuated oligomer-stimulated cytokine secretion demonstrating that the oligomer stimulated response required tyrosine kinase activation. In order to correlate the in vitro oligomer-stimulated microgliosis with human disease, AD and age-matched control brains were analyzed via Western blot analysis for changes in levels of tyrosine phosphorylated proteins and active Src family members, Lyn and Src. End stage diseased brains did not demonstrate quantitative differences in tyrosine phosphorylated protein levels or active levels of Src kinases correlating with the possibility that the oligomeric species of AB has a larger role in earlier disease stages. Based upon the FDA approval status of dasatinib for lymphoblastic leukemia, this drug or a related tyrosine kinase inhibitor may prove useful in attenuating oligomer-dependent microgliosis and proinflammatory changes in the brain.

# FUSARIUM SPECIES ASSOCIATED WITH ROOT ROT OF DRY BEANS IN NORTH DAKOTA

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North Dakota is the largest producer of dry edible beans (*Phaseolus vulgaris L.*) in the United States with 660,000 acres planted to this crop in 2008 (USDA-NASS). The ability of dry beans to fix atmospheric nitrogen and their high nutritional value has made them popular in rotations with cereals. However, diseases are a major threat to dry bean production and root rots rank among the most important diseases affecting bean production areas in North Dakota and adjoining Minnesota. Root rots in dry beans are known to be caused by a complex of several fungal species among which, Fusarium solani f. sp. phaseoli and Rhizoctonia solani were considered to be the most common in this region according to previous reports (Jensen et al., 2002). Due to rise in disease incidence, the pathogen population involved in causing root rots was revaluated in a survey conducted during the summers of 2007 and 2008, covering major dry bean growing counties of North Dakota. Pathogens were isolated from symptomatic roots using standard microbiological methods and initially identified by morphological characteristics. This was followed by PCR and sequencing of either the translation elongation factor one alpha or the internal transcribed spacer regions which were compared to available fungal identification databases. Fusarium species including F. solani, F. oxysporum, F. acuminatum, F. avenaceum, F. redolens and F. graminearum as well as Rhizoctonia solani were isolated from these samples. Apart from the known dry bean root rot pathogen F. solani f. sp. phaseoli, this study resulted in the isolation of F. graminearum and F. avenaceum, species generally associated with cereals from symptomatic dry bean roots. These two pathogens, though known to be able to cause root rot in dry beans (Chongo et al., 2001; Ares et al., 2006) have not been associated with this disease in North Dakota in the past. Two other species of particular relevance detected from the diseased roots were F. acuminatum and F. redolens. This would be a first report demonstrating the ability of these two toxigenic Fusarium species to infect dry beans. Isolates of F. acuminatum and F. redolens from dry beans were evaluated for their ability to cause root rot under controlled conditions and Koch's postulates established using a susceptible kidney bean variety. A host range study is in progress to assess the pathogenicity of these two newly found Fusarium species on different crops commonly grown in rotation with dry bean. The identification of the currently prevalent root rot pathogens and other findings from this study are crucial for developing disease management strategies and would greatly impact the dry edible bean industry in the state.

Key words: Fusarium, Root rot, Phaseolus vulgaris, Host Range.

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# PROTEOMIC PROFILING AND ANTIGENIC CHARACTERIZATION OF EXTENSIVELY-DRUG RESISTANT *ESCHERICHIA COLI* STRAINS ISOLATED FROM SCOURING CALVES

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Targeting drug efflux pumps (EPs) with chemical inhibitors has become a popular research objective aimed at finding efficacious treatment for extensively resistant (XDR) infections. However, only few studies have investigated antibodies as potential EP-blocking-agents that may potentiate antimicrobial sensitivity in XDR bacteria. The objectives of this research were: to assess the immunogenicity of components of the AcrAB-TolC efflux pump; and to identify and characterize previously unreported XDR-associated Escherichia coli proteins. These studies are needed as a preamble to the investigation of the potential use of anti-TolC serum as an EPblocking-agent that could provide basis for developing therapeutic products for treatment of XDR infections. Proteomes of XDR and antimicrobial sensitive (AS) E. coli were studied in 1-D and 2-D silver stained gels, and in Western blots (WBs). Liquid chromatography-mass spectrometry (LC-MS) analysis was performed on protein spots representing proteins of interest. Polyclonal sera strongly recognized a number of proteins (31, 33, 36, 62, and 80 kDa) plus many faint protein bands in all study E. coli isolates. In 2-D WBs, EF-Ts focused as multiple spots of small isoelectric point (pI) differences. Collective results of WB and LC-MS strongly suggest upregulation of TolC and OmpA, and downregulation of Dps, 30S ribosomal protein S1, and EF-Tu in XDR strains. Results of WB alone suggest upregulation of 36 and 80 and downregulation of 42 kDa proteins in XDR strains. Anti-TolC serum also recognized 2 additional proteins of 36 and 62 kDa; with the former evidently staining strong in 2/3 of the XDR and 1/2 of the AS isolates. Based on MW, the 36 kDa, 42 kDa and 80 kDa proteins correspond to OmpC, OmpF and FhuA respectively. TolC, OmpA and OmpC have been cited for their role in drug resistance in E. coli. This study demonstrates that these proteins may play important roles in mediating XDR phenomenon in E. coli. Downregulation of OmpF, Dps and 30S ribosomal protein might also be associated with XDR phenomenon. Our study shows that, these proteins may be potential targets for immunomodulation of antimicrobial sensitivity in MDR pathogens.

# ALPHA-1A ADRENERGIC RECEPTOR ACTIVATION ENHANCES NEUROGENESIS AND COGNITIVE FUNCTION

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A major target of the adrenergic system is the hippocampus, a region that is critical for learning and memory. Recent studies suggest alpha-1A adrenergic receptors (a1A-ARs) may regulate neurogenesis and neuronal differentiation. Our understanding of the function of a1A-ARs is limited due to a lack of specific ligands and antibodies. To address this, transgenic mice were generated which over-express the a1A-AR with enhanced green fluorescent protein (EGFP) or constitutively active mutant (CAM) a1A-AR. Knockout (KO) a1A mice were also generated. Immunohistochemistry showed that CAM ala-AR mice had increased BrdU incorporation compared to normal and KO a1A-AR mice. Increased numbers of hippocampal interneurons in CAM α1A mice compared to normal mice were also observed. Increased interneurons may affect learning and memory. Normal, CAM α1A, and KO α1A mice were tested on a multi-component T-maze and the Morris water maze. CAM α1A mice displayed increased cognitive ability in these mazes compared to normal mice. In both models, KO a1A-AR mice displayed the worst cognitive ability. Treating normal mice with the selective a1A-AR agonist cirazoline also showed enhanced learning and memory processes. Stimulation of α1A-ARs may offer a new therapeutic strategy for increasing cognitive function and treating neurodegenerative diseases.

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### THE ROLE OF B1 AND B2 CELLS IN ALLERGIC ASTHMA

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Asthma is a chronic inflammatory disease of the lungs which affects more than 200 million people worldwide. In the United States alone, asthma affects more than 20 million people and is the most frequent cause of childhood hospitalization. Asthma is characterized by reversible airway obstruction that is associated with inflammation and airway hyperresponsiveness. Allergic asthma may result in chronic, irreversible remodeling of the bronchial architecture as indicated by increased smooth muscle and fibrosis around the airways, which is aggravated by mucus hypersecretion into the airway lumen.

B cells play a critical role in mucosal immunity, and we hypothesize that they also play an important role in the allergic airway. Using a murine fungal aeroallergen model to mimic human asthma, we have tracked the presence of T and B lymphocytes as well as plasma cells in the lung during the progression of the disease. As expected, these cells are recruited into the airways after allergen challenge. IgE and IgA were prominent in the serum and bronchoalveolar lavage fluid of allergic animals, suggesting both a systemic and tissue-focused production. Goblet cells producing copious amounts of mucus were noted in the allergic lungs particularly at day 7 after challenge, which coincided with peak IgA levels in the lumen of the airways. Planned research will determine the specificity of systemic and tissue-specific antibodies produced after allergen challenge, as well as the capacity for pro-allergy cytokine production and antigen presentation by B cells. With a firm understanding of the range of B cell function in allergic asthma, we assert that the selection of candidate targets will be better informed to provide more efficacious therapeutic drug interventions.

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# EFFECTS OF MATERNAL NUTRITION AND SELENIUM STATUS ON OFFSPRING CUTOUT.

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### North Dakota State University, Fargo

To determine impacts of maternal undernutrition or overnutrition, high Se, or a combination on lamb composition in finished lambs. 82 pregnant Rambouillet ewe lambs were allotted randomly to 1 of 6 treatments in a 3 x 2 factorial design that included plant of nutrition (60% [RES], 100% [CON], and 140% [HIGH]) and dietary levels of Se (adequate Se [ASe; 7.4 µg/kg BW] vs. high Se [HSe: 85 µg/kg BW] from enriched yeast). Treatments were initiated at breeding for Se and d 40 of gestation for nutrition. Pelleted diets were fed once daily (36.5% beet pulp, 22.3% alfalfa meal, 16.2% corn, 18% soybean hulls, and 7.0% soybean meal; 14.4% CP, 2.63 Mcal ME/kg; DM basis). Immediately after parturition, all lambs were removed from dams and fed commercial colostrum and conventionally raised to approximately 60 kg. After harvest, lamb tissues were dissected into subgtaneous fat (SubQ), internal fat (perirenal), seam fat, dissected lean, bone, semimembranosus muscle (SM), and Psoas major muscle (PsM) and weights recorded. No differences were observed in carcass weights or USDA Yield Grade. However, dissected lean (p = 0.0005), SubQ fat (p = 0.003), seam fat (p = 0.02), and bone (p = 0.006) was significantly greater in lambs that were born as singles vs. those that were born twin. Males had more SubQ fat (p = 0.008), more bone (p = 0.0002) than females. Lambs whose mothers were fed selenium had more perirenal fat (p = 0.007) and heavier Psm weight (p = 0.07) and lambs whose mothers were fed 140% plane of nutrition had lighter SM and PsM weight compared to lambs whose mothers were fed 60% and 100% plane of nutrition.

# THE FIVE TRANSMEMBRANE VASOACTIVE INTESTINAL PEPTIDE RECEPTOR 1 ISOFORM IS EXPRESSED IN HUMAN BLOOD CD14+ MONOCYTE CELLS

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Vasoactive intestinal peptide receptor 1 (VPAC1) is a 7 transmembrane (TM) G protein coupled receptor that couples to Gαs and increases intracellular cAMP levels in response to external stimuli. Recently, a novel VPAC1 isoform was identified in human peripheral blood mononuclear cells (PBMC) that lacks exons 10-12 to form a 5TM receptor that lacks Gαs coupling, but elicits significant tyrosine phosphorylation when treated with exogenous ligand in ectopically overexpressed CHO cells. VPAC1 is a deactivating factor for peripheral blood monocytes by inhibiting the production of many proinflammatory cytokines, but fails to increase intracellular cAMP levels. We hypothesize dual expression of 5 and 7TM VPAC1 may act antagonistically with regard to intracellular cAMP elevation. Uncovering the expression profile of the 5TM VPAC1 splice variant is a necessary first step at understanding the biological role of this novel receptor isoform. To this end, mRNA was collected from CD14+ cells separated from human PBMC by magnetic beads. First strand cDNA was synthesized with anchored primers and the expression profile of the 7TM and 5TM assessed by RT-PCR, sequence analysis, and Southern hybridization. Our findings suggest preferential 5TM expression in blood monocytes (CD14+).

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## DETECTION OF DNA SEQUENCES USING GOLD NANOROD ENHANCED FLUOROPHORES

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Gold nanorods (AuNRs) have become an important type of metallic nanomaterial in recent years. As labeling nanomaterials, AuNRs have been used in a wide variety of biological applications. In this work, we employed AuNRs as substrates to enhance the fluorophore AlexaFluor 750 for DNA detection. The fluorescence intensity of AlexaFluor 750 dye molecules was enhanced by the surface plasmon resonance (SPR) of gold nanorods.

The nanorods have shown a good stability in a number of environments, including various pH solutions, over a lengthy period of time, and with different concentrations of ligands attached on the surface. The DNA detection was carried out through a sandwich assay. First, a thiolated DNA strand (5' HS-TTT TTT ATT CGG TCA GCT 3') was immobilized on the surface of the AuNR. Then, this capture DNA hybridizes with the target DNA (3' TAA GCC AGT CGA TAC ACC CCG GAG AAG 5'). A probe DNA (5' ATG TGC GGC CTC TTC 3') was first labeled with AlexaFluor 750 and then was hybridized with the target DNA to form a sandwich assay. The fluorescence enhancement is proportional to the amount of capture DNA tethered to the AuNRs. Due to the emission wavelength in the near-infrared region, the assay has a great potential for many biological applications.

# α<sub>1A</sub> ADRENERGIC RECEPTOR ENHANCEMENT OF NEUROGENESIS AND INTERNEURON FUNCTION

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Adrenergic receptors (ARs) have been shown to regulate neuronal function in diverse ways, from altering membrane potential and transmitter release to effects on gene expression. It has been shown that  $\alpha_1AR$  activation produces a membrane hyperpolarization in a specific subset of hippocampal interneurons. Evidence from transgenic mice either lacking (KO) or overexpressing (CAM) one or more  $\alpha_1ARs$  suggest that the majority of this response is due to activation of  $\alpha_{1A}ARs$ . Electrophysiological recordings during application of  $\alpha_{1}AR$  agonists produced a concentration-dependent increase in action potential frequency in hippocampal interneurons in normal and  $\alpha_{1B}AR$  KO mice, but not in  $\alpha_{1A}AR$ -KO mice. Of particular interest, mice overexpressing the α<sub>1A</sub>AR showed increased hippocampal interneuron density. BrdU immunohistochemistry revealed enhanced neurogenesis in CAM  $\alpha_{1A}AR$  animals. This result was duplicated in wild type mice treated with the  $\alpha_{1A}AR$  agonist, cirazoline. When treated with the epileptogenic agent flurothyl and compared to controls, CAM  $\alpha_{1A}$  AR mice showed an increase in latency periods preceding seizures. These findings suggest that activation of  $\alpha_{1A}ARs$  increases inhibitory tone not only via increased interneuron firing but potentially by increased numbers of interneurons. These findings are potentially very significant because they link  $\alpha_{1A}AR$ -induced proliferation of interneurons to the antiepileptic actions of the adrenergic system.

Supported by NIH, NIH COBRE, NSF ND EPSCoR, NSF CAREER, Epilepsy Foundation.

### FUNGAL VIABILITY INFLUENCES THE IMMUNE RESPONSE IN ALLERGIC ASTHMA

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Allergic asthma is a chronic pulmonary syndrome in which inflammatory cells and their mediators initiate a sequence of events that result in narrowed airways and make breathing difficult. Nearly 16.8 million adult Americans have been reported to suffer from asthma. Epidemiological studies have linked sensitization to fungal spores with the severity of asthma. Aspergillus fumigatus is one such clinically relevant fungal allergen ubiquitously found in the environment. Unlike pollen and pet dander, A. fumigatus spores (conidia) have the ability to grow and dwell inside the lungs. Moreover, they can secrete proteases which may interact with the host's structural and immune cells and potentially influence the pathology of asthma. Our aim in this study was to determine the extent to which the viability of A. fumigatus conidia and their associated ability to produce proteases, is necessary in the development of allergic inflammation.

**Method:** BALB/c mice sensitized to soluble *A. fumigatus* antigens were challenged with either live or irradiation-killed mature aerosolized conidia. On days 1 and 28 post-challenge, serum IgE and airway hyperresponsiveness (AHR) were used to assess allergic asthma. Morphometric identification and counting of the leukocytes was performed on the bronchoalveolar lavage samples following cyto-centrifugation and staining. Histological analysis was used to measure the thickness of subepithelial collagen deposition from day-28 lung sections.

Results: Both live and irradiation-killed conidia produced allergic airways disease, as assessed by similar serum IgE and AHR levels (data not shown). Although, the level of inflammation elicited by both; live and irradiation-killed mature conidia was the same (Figurela), but the type of phagocytic cells recruited to the airways, on day 1 post challenge, was significantly different between the groups. Live conidia elicited a predominantly neutrophilic inflammation (Figurelb), while irradiation-killed conidia balanced neutrophila with a strong macrophage influx (Figurelc). Neutrophil and macrophage levels between the two groups were compared using Welch's corrected t test (GraphPad InStat software). Both live and killed conidia treatments resulted in increased collagen deposition when compared to naïve animals, but there was no significant difference between the two treatment groups (data not shown).

**Conclusion:** Mold-associated exposures are a serious concern following post-flood and post-hurricane situations, as well as chronic exposure to damp indoor settings or outdoor environments. In our study, we showed that inflammatory response in allergic mice can be influenced by the metabolic state of *A. fumigatus* conidia. Although inflammation, IgE, AHR, and collagen deposition was largely the same between the groups, the cells recruited to the airways were different. Live conidia elicited a strong neutrophil influx and irradiation-killed conidia balanced neutrophilia with macrophages. Neutrophilic inflammation has been linked to steroid resistance in individuals with stable asthma, although the mechanisms for steroid resistance are not known. The differential recruitment of inflammatory cells may have a major impact on treatment strategies for mold-associated asthma. Ongoing research in this area is expected to aid in understanding the mechanisms involved and therapeutics available for the treatment of allergic diseases.

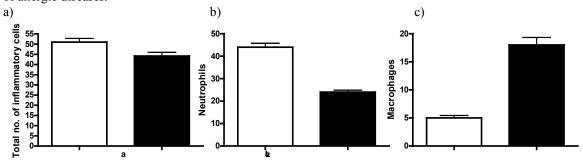


Figure 1: BAL leukocyte counts in *A. fumigatus*-sensitized mice at day 1 after airborne conidia (live or irradiation-killed) challenge. Although the total number of inflammatory cells (a) was the same between the two groups, neutrophils (p<0.0001) (b) and macrophages (p=0.0003) (c) were significantly different. Data are expressed as the mean number of cells per hpf±SEM; n=5 mice/group.

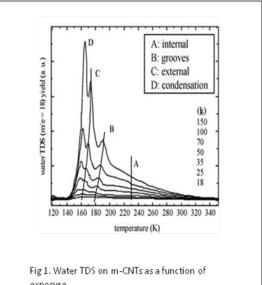
## POSSIBLE EFFECT OF CARBON NANOTUBE CRYSTAL STRUCTURE ON GAS-SURFACE INTERACTIONS-THE CASE OF BENZENE, WATER AND N-PENTANE **ADSORPTION ON SWCNTS**

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Thermal desorption spectroscopy (TDS) technique was used to investigate the effect of carbon nanotubes (CNT) crystal structure on adsorption kinetics of benzene, water, and npentane (1). The kinetics of adsorption of these molecules on single wall carbon nanotubes (SWCNTs) such as metallic CNTs (m-CNTs), semiconducting CNTs (s-CNTs, brand name CoMoCAT) and mixed CNTs(brand name HiPco) was studied. Scanning electron microscopy (SEM) images of these samples were obtained from Argonne national laboratory. Metallic CNTs were obtained from Clemson University.

Theoretically it is predicted that the binding energies increase with increase in CNT diameter for non covalent interactions. For covalent interactions it is predicted that the binding energies for adsorption on the inner CNT surface decrease with increase in CNT diameter and binding energies on the outer surface are smaller and independent of CNT diameter.

The diameters of CNTs used in this study decreases from 1.4 nm to 0.8 nm (m-CNTs > HiPco s-CNTs). For all the CNT samples studied, four distinct features are found in each set of TDS curves. The peaks are labeled as A, B, C which refer to probe molecule from adsorption/desorption of



exposure.

internal, groove and external sites respectively. Peak D is an adsorption unspecific condensation peak. Interestingly for benzene, a trend of slightly decreasing binding energy with increasing CNT diameter is seen for adsorption on internal sites and a much larger heat of adsorption (E<sub>d</sub> = 80kJ/mol) has been obtained for the these sites. This is in contrast to theoretical predictions (E<sub>d</sub>= 19kJ/mol). In agreement with DFT calculations, a very small increase in binding energies (0.9 kJ/mol) with increasing CNT diameter is seen for adsorption on external and groove sites. For npentane and water, no clear correlation of E<sub>d</sub> with CNTs crystal structure is evident.

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Reference: Possible effect of carbon nanotube crystal structure on gas-surface interactions – the case of benzene, water, and n-pentane adsorption on SWCNTs at ultra-high vacuum conditions

M. Komarneni, A. Sand, J. Goering, U. Burghaus, M. Lu, L. Monica Veca, Ya-Ping Sun, submitted

# EFFECT OF MATERNAL DIET ON FETAL:MATERNAL RATIO OF CIRCULATING AMINO ACIDS, NON-ESTERIFIED FATTY ACIDS, BLOOD UREA NITROGEN, AND GLUCOSE CONCENTRATIONS IN EWES

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We hypothesized the previously reported reduction in fetal wt, without a reduction in placental wt, was achieved by decreased nutrient transport to the fetus. Therefore, the objectives were to determine the effects of maternal diet on the fetal:maternal ratio of circulating glucose, amino acids, NEFA, and BUN concentrations. Pregnant ewe lambs (n = 54) were assigned to 1 of 8 treatments in a 2 x 2 x 2 factorial design: selenium (Se) level [initiated at breeding; adequate (ASe; 0.3 ppm Se) or high (HSe; 3.0 ppm Se)] and nutritional level [control (C) or restricted (R); fed to meet 100% or 60% of NRC recommendations] fed at different times of gestation [Mid (d 50 to 90) or Late (d 90 to 130)]. Blood samples were taken from ewes and fetuses on d 130 of gestation. Glutamate, glutamine, serine, and glycine were analyzed in plasma samples, and glucose, NEFA and BUN were analyzed in serum samples. A Se x Late interaction showed the fetal:maternal ratio of glutamate was greater (P = 0.03) in HSe-C compared to ASe-C and HSe-R (2.39 vs. 1.49 and 1.74  $\pm$  0.32) with ASe-R ewes being intermediate. A Se x Late interaction indicated a greater (P = 0.03) fetal:maternal ratio of glutamine in ASe-R compared to ASe-C and HSe-C ewes (2.71 vs. 1.31 and 1.88  $\pm$  0.33). The Late-R group exhibited greater (P = 0.02) serine fetal:maternal ratio compared to Late-CON ewes (9.94 vs.  $6.43 \pm 1.07$ ). There was a Se x Late interaction on glycine, with ASe-R having a greater (P = 0.03) fetal:maternal ratio compared to all other ewes (1356.61 vs. 1027.64, 1159.92, and 1192.66  $\pm$  74.56 for ASe-C, HSe-C, and HSe-R, respectively). The Late-R ewes had a lower (P = 0.01) NEFA fetal:maternal ratio compared to the Late-C ewes (0.12 vs.  $0.19 \pm 0.02$ ). The BUN fetal:maternal ratio was lower (P = 0.01) in the HSe compared to the ASe ewes (1.04 vs. 1.19  $\pm$  0.04). The fetal:maternal glucose ratio did not differ ( $P \ge 0.14$ ). These data indicate the fetal to maternal concentration gradient of certain amino acids, NEFA, and BUN are affected by Se and nutritional level during late gestation, indicating a role for maternal diet impacting placental function.

# IN SITU GROWTH OF AU NANORODS ON TIO<sub>2</sub> SURFACES FOR PHOTOELECTROCHEMICAL SOLAR CELLS

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Au nanorods represent interesting one-dimentional (1D) nanostructures that have been loaded on various surfaces through Langmuir-Blodgett (L-B) method, chemical templating, etc. Since the resonance wavelength of Au nanorods are highly dependent on their aspect ratios, the unique plasmon absorbance features have been exploited for a wide variety of applications such as chemical and biosensors.

In this work, we described a new method to in situ synthesize Au nanorods on a  $TiO_2$  surface. It took advantage of the catalytic property of  $TiO_2$  to reduce the  $[AuCl_4]^-$  ions anchored on a  $TiO_2$  nanocrystal surface to the  $Au^0$  nanoseeds under the irradiation of UV light. Then, the film was immersed in a surfactant-containing solution, inducing the growth of Au nanoseeds into a rod shape. The aspect ratios of Au nanorods were determined by the composition of the Au growth solution. The nanocomposite film was exploited in a photoelectrochemical solar cell, and the results showed that the photocurrent was significantly enhanced compared to the pure  $TiO_2$  film, that might be because the presence of Au nanorods suppressed electron-hole recombination at the interface.

# CHARACTERISATION OF ANTIMICROBIAL RESISTANCE (AMR) AND PRESENCE OF CLASS 1 INTEGRONS IN *SALMONELLA* SEROVARS ISOLATED FROM CLINICAL CASES OF ANIMALS AND HUMANS IN NORTH DAKOTA.

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Salmonella has been cited as one of the leading causes of food borne illness world wide and in the United States (USA) (2), and as an indicator organism for studying antimicrobial resistance (AMR) trends (3). The objective of this study was to characterise AMR patterns of Salmonella isolates from clinical cases of animals and humans in North Dakota, USA and determine the association between the observed AMR and presence of class 1 integrons. Isolates were collected from the Veterinary Diagnostic Laboratory (VDL) at North Dakota State University and the North Dakota Department of Health, respectively between 2003-2007. Culturing and characterisation was done according to methods optimised for Salmonella detection. AMR profiles were determined using a panel of 15 antimicrobials as per the manufacturer's instructions (Sensitire, Trek Diagnostics System, and Westlake, Ohio). Screening for the class 1 integrons was done using PCR with primers specific for the int1 (1).

Out of 248 Salmonella isolates tested 22% (55/248) and 37% (92/248) were resistant to  $\geq$ 5 antimicrobials and at least 2 antimicrobials, respectively. For cattle and human isolates 48% (37/77) and 11% (18/171), respectively, had resistance to  $\geq$ 5 antimicrobials while 64% (49/77) and 25% (43/171), respectively, were resistant to 2 or more antibiotics. Pan susceptible isolates were 14.3% (11/77) in cattle and 19.3% (33/171) in humans. A statistically significant difference (p<0.05) in the AMR patterns present in humans and cattle was observed for all drugs except Trimethoprim, Nalidixic acid, Gentamicin, Ciprofloxacin and Amikacin. The highest resistance frequency was seen against Tetracycline in both cattle (61% (47/77) and humans (21.1%) (36/171) followed by Chloramphenical 58.4% (45/77) in cattle and 21.1% in humans (36/171). Multidrug resistance (MDR) was observed in both animal and human isolates (Figure 1).

Although only one isolate was resistant to ciprofloxacin (a drug of choice for treatment of *salmonellsosis* in humans), (5.2%) 4/77 cattle and (5.9%) 10/171 human isolates were resistant to nalidixic acid, a drug in the same class with Cipro; This indicates a possible emergence of treatment failure due to induced resistance of *Salmonella* spp. towards ciprofloxacin. Among cattle isolates, 25% (12/48) tested positive for presence of integron 1. Also, presence of integron 1 was significantly associated (OR 0.18, 95% CI 0.043, 0.9347, P value Fishers exact 0.0254) with AMR to ceftiofur. These preliminary results indicate that higher resistance was observed against antibiotics

widely used in veterinary medicine. Resistance was also observed against drugs whose veterinary use is restricted, implying possible horizontal transmission, mediated by molecular structures such as class one integrons. More studies need to be done to determine other mechanisms involved in explaining AMR patterns of *Salmonella* isolates from different sources and determine their clonal relatedness.

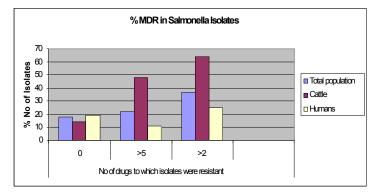


Figure 1: Multidrug resistance (MDR) in Salmonella isolates from animals and humans in ND. (N) Total population= 248; Cattle, N = 77; Humans, N= 171

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## MULTIPLEX ELECTROCHEMICAL IMMUNOASSAY USING GOLD NANOPARTICLE PROBES AND IMMUNOCHROMATOGRAPHIC STRIPS

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We describe a multiplex electrochemical immunoassay based on the use of gold nanoparticle (Au-NP) probes and immunochromatographic strips (ISs). The approach takes advantage of the speed and low cost of the conventional IS tests and the high sensitivities of the nanoparticle-based electrochemical immunoassays. Rabbit IgG (R-IgG) and human IgM (H-IgM) were used as model targets for the demonstration of the proof concept. The Au-NPs based sandwich immunoreactions were performed on the IS, and the captured gold nanoparticle labels on the test zones were determined by highly sensitive stripping voltammetric measurement of the dissolved gold ions (III) with a carbon paste electrode. The detection limits are 1.0 and 1.5 ng ml with the linear range of 2.5–250 ng ml for quantitative detection of R-IgG and H-IgM, respectively. The total assay time is around 25 min. Such multiplex electrochemical immunoassay could be readily highly multiplexed to allow simultaneous parallel detection of numerous proteins and is expected to open new opportunities for protein diagnostics and biosecurity.

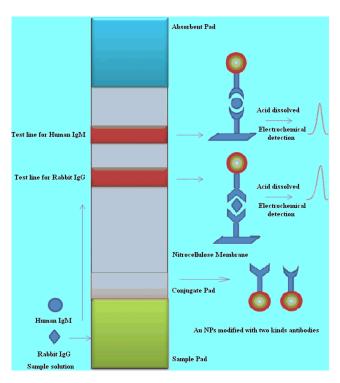


Fig. 1. Schematic illustration of multiplex electrochemical immunoassay based on the immunochromatographic test strip and gold nanoparticle labels.

Mao, X, Baloda, M, Gurung, A, Lin, Y, Liu, L, Electrochemistry Communications, Volume 10, Issue 10, October 2008, Pages 1636-1640

## ROUGH CALCULATIONS PERTAINING TO SCANNING TUNNELING MICROSCOPY AND ANGLE RESOLVED PHOTOEMISSION SPECTROSCOPY ON GRAPHENE

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The term graphene refers to a single layer of graphite, or carbon in honeycomb form (1). Although the idea has been around for some time, preparation of and experimental work (2) on actual graphene sheets is new. Graphene is a degenerate semimetal that may become important for electronic devices due to high carrier mobility (3) and because it is only a monolayer thick. It is also relatively inexpensive. Perhaps more importantly, its peculiar band structure affords a physical realization of a two-dimensional system of mass-less spin ½ Fermions. Such systems have long fascinated high-energy theorists.

In this note we report a crude model Green-function calculation of graphene electronic properties within the Hückel  $\pi$ -orbital theory. Specifically, we show local density of states (LDOS) measurable by scanning tunneling microscopy (STM) and local spectral densities relating to angle-resolved photoelectron spectroscopy (ARPES) of carbon atoms near the zig-zag edge in graphene.

The LDOS shown in Fig. 1 is density of  $\pi$ -orbital states, projected onto a particular atom at the edge. Figure 2 shows a density of states projected onto both crystal momentum component k parallel to the edge and the distal row of edge atoms. The zig-zag edges are apt to form as lattice imperfections (2) in the graphene sheets. Both figures show the edge state believed to form at the vertex of the Dirac cone where filled and empty  $\pi$ -orbitals meet at zero energy.

Figure 1.  $\pi$ -orbital LDOS for a distal site on a zig-zag edge of graphene. Dimensionless units are based on band width and atom spacing.

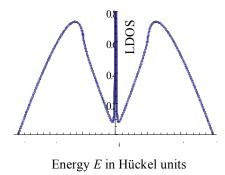
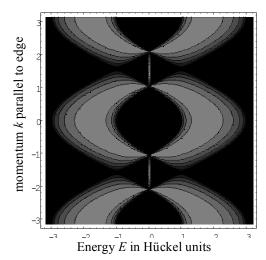


Figure 2. Contour plot of spectral density.



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# RGS7 PROTEIN SUPPRESSION OF $G\alpha_0$ Protein-Mediated $\alpha_{2A}$ -ADRENERGIC RECEPTOR INHIBITION OF MOUSE HIPPOCAMPAL CA3 EPILEPTIFORM ACTIVITY

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G-protein coupled  $\alpha_2$  adrenergic receptor (AR) activation by epinephrine (EPI) inhibits epileptiform activity in the mouse hippocampal CA3 region. The mechanism underlying this action is unclear. This study investigated which subtypes of  $\alpha_2ARs$ , G-proteins ( $G\alpha_0$  or  $G\alpha_i$ ), and RGS proteins were involved in this response using recordings of CA3 epileptiform bursts in mouse brain slices. First, we determined this effect was mediated by the  $\alpha_{2A}AR$  subtype as the inhibitory action of EPI on epileptiform burst frequency was abolished in slices from  $\alpha_{2A}AR$ , but not  $\alpha_{2C}AR$ , knockout mice. Next, using transgenic mice with the G184S Gnai2 allele (knock-ins) which interrupts G-protein  $\alpha$  unit binding to regulators of G-protein signaling (RGS), we found  $\alpha_{2A}AR$  antiepileptic effects of EPI were enhanced in hippocampal slices from mutant  $G\alpha_0$  mice but not  $G\alpha_{i2}$  mice. Finally, knockout mice for the RGS7 protein family were found to have increased  $\alpha_{2A}AR$ -mediated hippocampal actions compared to their littermate controls. These results indicate that the EPI-mediated inhibition of mouse hippocampal CA3 epileptiform burst activity is through an  $\alpha_{2A}AR/G\alpha_0$ -mediated pathway under strong inhibitory control by proteins of the RGS7 family. This suggests a possible role for selective  $\alpha_{2A}AR$  agonists or RGS7 inhibitors as a novel antiepileptic drug therapy.

Supported by the American Physiological Society, ND EPSCoR EPS-0447679, NSF 0347259, NSF 0639227, NIH P20RR0167141, NIH 5RO1DA17963 and NIH 5RO1GM039561.

Lymphocytes (average cells/HPF)

100

Figure 1. Lymphocytes

peaked in the allergic

lungs 7 days after fungal

challenge. \*\*p<0.01

### ANTIBODY PRODUCTION IS PROMOTED IN THE ABSENCE OF VPAC-2 RECEPTOR IN A MURINE MODEL OF ALLERGIC ASTHMA

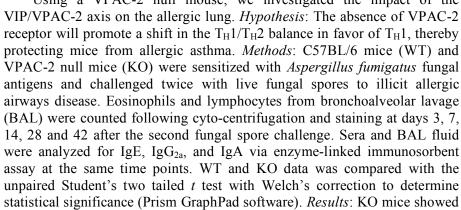
### Amali Samarasinghe\*, Scott Hoselton, and Jane Schuh

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Evidence gathered over the past few decades emphasize the importance of interaction between the neuroendocrine and immune systems in homeostatic organ function and development. Alterations in this bi-directional communication can lead to the pathogenesis of various diseases. Acting through its Gprotein coupled receptors, VPAC-1 and VPAC-2, vasoactive intestinal peptide (VIP) functions to modulate the innate and adaptive arms of the immune system. VIP produced by lymphocytes in response to various stimuli such as inflammation, proliferation, and antigen exposure has recently been shown to promote the differentiation of helper T cells favoring a T<sub>H</sub>2 phenotype with survival and generation of memory T<sub>H</sub>2 cells. Allergic asthma is a T<sub>H</sub>2 mediated disease of the airways characterized by airway inflammation, goblet cell metaplasia, smooth muscle cell hyperplasia, angiogenesis, and fibrosis.

Although VIP has been studied in the context of various diseases, its possible role in asthma has not been explored to its full potential.

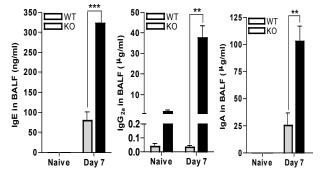
Using a VPAC-2 null mouse, we investigated the impact of the



a delay and reduction in eosinophils compared to WT controls, but increased lymphocyte egress into the airway lumen peaking at day 7 (Fig 1). The peak in lymphocytes in the airways corresponded with the peak in antibodies in the KO. Elevated basal serum IgG<sub>2a</sub> and IgA were characteristics that distinguished the VPAC-2 null mouse from their WT counterparts. The BAL fluid of allergic KO mice contained

significantly more IgE, IgG<sub>2a</sub>, and IgA than the WT control following allergen challenge (Fig 2).

Conclusions: VPAC-2 may play a role in eosinophil recruitment since its absence reduced eosinophil influx into the lung. The trend in antibody availability in the BAL fluid correlated with the trend in lymphocytes implying that the allergen induced infiltration of lymphocytes may contain T cells that can activate B cells in situ. VIP may mediate positively antibody production VPAC-1 in a T-dependent manner and the presence of VPAC-2 may hinder this effect. Although IgG<sub>2a</sub> and IgA were abundantly produced in the KO mice, the presence of



**Figure 2.**  $T_H2$  (IgE) and  $T_H1$  (Ig $G_{2a}$  & IgA) type antibodies peaked in the airways at day 7 in both groups, with KO mice producing more antibodies than the WT. \*\*p<0.01, \*\*\*p<0.001

these T<sub>H</sub>1 antibodies did not protect mice from A. fumigatus induced allergic asthma.

## CHARACTERIZATION OF THE 2007 OUTBREAK OF NEPHROTOXICITY AMONG DOGS AND CATS ASSOCIATED WITH MELAMINE IN PET FOOD

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**Objective.** To characterize the 2007 outbreak of renal failure in dogs and cats associated with melamine in pet food; by clinical signs, severity of disease and spatial and temporal distribution of the pets affected. **Design.** Retrospective case series.

**Data Source and Analysis.** Through Freedom of Information Act (FOIA) data were obtained from the US Food and Drug Administration (FDA) on pet foods, pets and pet owners affected nationwide during the 2007 pet food recalls. A *confirmed case* of nephrotoxicity in a dog or cat was defined as one with at least three of the typical clinical signs and symptoms of nephrotoxicity, consumed a recalled product and visited a veterinarian; while *a probable case* was defined as one with at less than three of the typical clinical signs and symptoms of nephrotoxicity and consumed a suspected product; and *a possible case* was defined as one that consumed a suspected product but did not show any clinical sign of nephrotoxicity. Data were entered in an excel spreadsheet and descriptive statistics run using SPSS version 7. EPI INFO version 3.2 was used to draw trends of the outbreak and map out the distribution of cases by state and by time of report. Using the fate of the dog/cat (whether died, had life threatening condition or recovered) as an outcome variable, chi square and logistic regression will be run to determine which variables were significantly associated with the fate of the animals.

Results. The 2007 melamine related nephrotoxicity in dogs and cats included over 10,409 cat and dog cases nationwide from almost all states with 2% confirmed cases, 65% probable and 43% possible cases. Almost half (44%) of the complaints to the FDA were defined by FDA as baseless, 40.4% perceived to be due to kidney disease, 4.8% associated with death of an animal (not attributed to kidney disease) and 8.7% suspected to have been due to pet food contamination. A total of 54% of dogs and cats exposed to melamine contaminated feed were reported to have died, 29% resulted in life threatening condition and 13.5% in non-life threatening condition. Over three-quarters (78%) of the respondents reported visiting a medical personnel/veterinarian of which 54% reported death of their dog or cat and 34% reported their dog or cats had a life threatening condition. The cats and dogs that died were however similar in the following characteristics (severity of illness and the pet food consumed) to those that did not die. Over 279 pet brands were contaminated, however 'lams' (20.3%) was the most affected, followed by 'Special kitty' (13.7%), 'Nutro' (11.4%), 'Ol Roy' (9.8%), 'Purina' (4.7%), 'Alpo' (4.4%) and others (34.6%). Chi square and logistic regression analyses are pending.

**Conclusion.** These data provide a better understanding of the epidemiology of the 2007 melamine related nephrotoxicity outbreak in the US and are vital for planning management strategies for similar future outbreaks

Figure 1: Distribution of nephrotoxicity cases reported in the US by time of reporting (N=10,409)

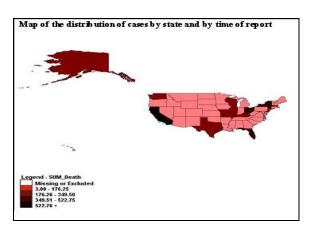
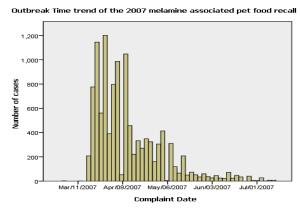


Figure 2: Outbreak time trend of the 2007 melamine associated pet food recall (N=10,409)



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## THE PALEONTOLOGY AND BIOSTRATIGRAPHY OF THE TURTLE BUTTE FORMATION, TRIPP COUNTY, SOUTH DAKOTA

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**Introduction** – The Turtle Butte Formation, exposed on Turtle Butte, in Tripp County, South Dakota, has yielded a diverse paleofauna. The Turtle Butte Formation and Wewela local fauna were first described in 1968 (1). Following the initial description, field crews from the South Dakota School of Mines and Technology collected fossil specimens and fossiliferous matrix in 1969 and 1973. However, the specimens collected in 1969 and 1973 were never identified until this project and are referred to here as Turtle Butte Assemblage. In 2004, Bailey (2) conducted bulk matrix sampling that yielded 17 microfaunal specimens. Skinner *et al.* (1) suggested that the megafauna of the Wewela local fauna of the Turtle Butte Formation is equivalent to the uppermost Monroe Creek or lowermost Harrison assemblages or both. Bailey (2), interpreted the fossils from the Turtle Butte Formation as late early Arikareean, although this age interpretation is based only on 17 specimens.

Results – The Turtle Butte Formation consists of approximately 22.5 m of pinkish to white sandy siltstone, claystone, and conglomerate beds. The Turtle Butte Formation was distinguished from the underlying Rosebud Formation by its white color and slightly larger grain size, and from the overlying Valentine Formation by smaller grain size and more homogeneous composition of sand-sized grains. The sediments of the Turtle Butte Formation were deposited in a fluvial system, specifically a meandering stream system evidenced by the conglomeratic sandstones of channel deposits and the large number of siltstone and claystone units representing floodplain deposits. The Turtle Butte Assemblage is diverse, with seven orders, although a bias against the megafauna is present. The assemblage includes: Hibbarderix sp., Proscalops sp. cf. P. secundus, Domnina sp., Ekgmowechashala sp., Gripholagomys lavocoti, Megalagus primitivus, Palaeolagus philoi, Palaeolagus sp., Alwoodia harkseni, Protosciuris sp., Protospermophilus sp. nr. P. kelloggi, cf. Nototamias sp., Palaeocastor large sp., Palaeocastor (Capatanka) large sp., Palaeocastor (Capatanka) small sp., cf. Capacikala sp., Pseudotheridomys sp. cf. P. hesperus, cf. Tenudomys sp., cf. Pleurolicus sp., Proheteromys sp., Schizodontomys sp., Sanctimus stuartae, Hitonkala macdonaldtau, Plesiosminthus grangeri, Paciculus nebraskensis, Nothocyon n. sp., Cynarctoides roii, Archaeohippus equinanus, and Merycochoerus superbus superbus.

**Discussion** – The Turtle Butte Assemblage has a diverse microfauna, particularly rodents and lagomorphs, typical of the Arikareean, as those orders were diversifying during that time. Surprisingly, the Turtle Butte Assemblage lacks mylagaulids and has few geomyid specimens, which are common in other late Oligocene-early Miocene assemblages. The Turtle Butte Assemblage has the greatest similarity to the Sharps and Monroe Creek formations in the Wounded Knee Area of South Dakota (3, 4). The Turtle Butte Assemblage also has the greatest similarity to other known late early Arikareean assemblages of South Dakota and northern Nebraska, such as the Skarboe Spur local fauna (2). The Turtle Butte Assemblage contains taxa representing the late early Arikareean Land Mammal Age, as determined using the identified assemblage and comparing it to known first and last appearances for the Arikareean as given in Bailey (2) and Tedford *et al.* (5). The Turtle Butte Assemblage has several taxa that have their first appearance in the late early Arikareean, including *Gripholagomys lavocati* and *Alwoodia harkseni*. The Turtle Butte Assemblage also has a taxon which makes its last appearance in the late early Arikareean, cf. *Tenudomys* sp.. *Hibbarderix* sp. from the Turtle Butte Assemblage represents a first appearance agerange extension from the late late Arikareean to the late early Arikareean. The new species of *Nothocyon* also represents a first appearance age range extension for the genus to the late early Arikareean from the early late Arikareean. Finally, the assemblage produces rare taxa, such as the primate *Ekgmowechashala*.

Conclusions – The Turtle Butte Formation of South Dakota has produced a diverse paleofauna. The Turtle Butte Formation was deposited by a meandering stream system evident by sandy siltstone and claystone floodplain deposits and conglomeratic paleochannel deposits. The assemblage is dominated by microfaunal elements and includes rare taxa, such as the primate, *Ekgmowechashala* and a new species of *Nothocyon*. The Turtle Butte Assemblage is determined to be late early Arikareean and is most similar to the Skarboe Spur and Monroe Creek local faunas of northern Nebraska and western South Dakota.

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## POSTTRANSLATIONAL REGULATION OF THE TUMOR-SUPPRESSOR IKAROS BY VASOACTIVE INTESTINAL PEPTIDE RECEPTOR 1 IN HUMAN HUT 78 CELLS

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Ikaros (IK) is a kruple-like zinc finger and master regulator of lymphopoesis. Mutations to the IK DNA binding domain result in T cell leukemia. The phosphorylated posttranslational modifications of IK have been shown to be regulated by casein kinase II (CKII) throughout the primary sequence. Putative consensus sequences for additional kinases such as GSK3, Cdk, CaMKII, and PKA have been identified. Hyperphosphorylation of IK reduces its DNA binding affinity and mediates G<sub>1/S</sub> transition. Vasoactive intestinal peptide/pituitary adenylyl cyclase activating peptide receptor 1 (VPAC1) is a G protein coupled receptor that negatively regulates T cell receptor (TCR) activation through a cAMP/PKA signaling dependent pathway when bound by its ligand, vasoactive intestinal peptide (VIP). Our hypothesis is that VIP/VPAC1 signaling suppresses CKII phosphorylation of IK in a PKA dependent mechanism. Upon T cell activation, a new immunoreactive pool of IK was detected, and VIP (10<sup>-8</sup>M) ablated the detection of this We conclude that VIP/VPAC1 signaling may block the hyperphosphorylated species. immunoreactive pool of a certain hyperphosphorylated IK species thereby negatively regulating T cell activation.

Research supported by NIH-K01 1K01DK064828 and COBRE 2P20RR05566.

## IN VIVO OPPOSING REGULATION OF VASOACTIVE INTESTINAL PEPTIDE RECEPTOR-1 AND -2 THROUGHOUT THE T CELL IMMUNE RESPONSE

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This study investigates vasoactive intestinal peptide receptor 1 (VPAC1) and VPAC2 regulation during the immune response of murine T cells. VPAC1 is an anti-proliferative, Gprotein coupled receptor that has been shown to be downregulated upon ex vivo T cell activation, whereas VPAC2 was shown to be upregulated. To date, regulation of VPAC1 and 2 have not been elucidated in primary, murine T cells throughout the phases of a T cell immune response following infection and whether they have a role in memory T cell evolution. Splenic CD4 T cells were isolated from C57Bl/6J mice 24hr post in vivo activation with anti-CD3. Flow cytometry (Accuri) analysis confirmed 90% activation of cells (CD69<sup>+</sup>/CD25<sup>+</sup>). QPCR analysis of total RNA showed VPAC1 levels decreased 93% in activated CD4 T cells, while VPAC2 levels increased 66%, possibly suggesting that these receptors regulate early components of the CD4 T cell response. These results prompted us to pursue an *in vivo* activation model utilizing OT-I and OT-II T cells. We hypothesize in CD4 T cells, VPAC1 levels will decrease upon activation and gradually increase to naïve levels throughout contraction into memory phase, while VPAC2 levels will increase upon activation and gradually decrease to lower naïve levels. The reciprocal expression pattern of these receptors may indicate they have opposing functions in regulating the kinetics of the T cell response and memory cell evolution.

1KO1 DK064828, COBRE- 2P20RR015566

## A NEW INTERPRETATION OF THE PALEOECOLOGY OF THE BIG PIG DIG QUARRY, BADLANDS NATIONAL PARK, SOUTH DAKOTA

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**Introduction** – In 1993, visitors found fossils exposed alongside Conata Road in the North Unit of Badlands National Park, South Dakota. These fossils became the first of many of what would become the Big Pig Dig Quarry. The Pig Dig Quarry is stratigraphically within the lower Scenic Member of the Brule Formation and is Orellan (~33Ma) in age, as indicated by the paleofauna. Ninety-five percent of the fossils are contained within a fossilbearing unit, called the "green layer," with the remaining five percent of the fossils within 5 cm above or below the "green layer." The site contains a large abundance of fossils representing numerous individuals with significant species diversity.

**Results** – During the 14 years of excavation, over 10,000 individual fossils have been removed from the quarry. These specimens are curated at the Museum of Geology Main Collections at the South Dakota School of Mines and Technology. The minimum number of individuals (MNI) for all taxa was calculated using the method outlined by Shotwell (1). At present, there are 75 individuals from 15 different genera. The MNI and corresponding genera are as follows (MNI Genus sp.): 22 *Archaeotherium* sp.; 19 *Subhyracodon* sp.; 13 *Leptomeryx* sp.; 10 *Mesohippus* sp.; 1 *Merycoidodon* sp.; 1 *Hypertragulus* sp.; 1 *Nimravus* sp.; 1 *Dinictis* sp.; 1 *Daphoenus* sp.; 1 *Palaeolagus* sp.; 1 *Megalagus* sp.; 1 *Ischyromys* sp.; 1 *Oropyctis* sp.; 1 *Stylemys* sp.; and 1 Aves order indet..

Discussion - The Pig Dig Quarry has been studied by a number of authors since the site's discovery. The earliest work produced a faunal list of four genera and stated the site was most likely a seasonal watering hole (2). As more field seasons produced more specimens, more genera were added to the fauna (3), but the watering hole interpretation was still accepted as recently as 2005 (4). The watering hole hypothesis was first proposed because the "green layer" was interpreted as an anoxic underwater environment, and the similarity between the hypothesized Oligocene and modern African climates (1, 2). With the calculated MNI presented here, the original hypothesis is problematic. A different interpretation may better account for the large number of predators/scavengers (carnivores). First, the abundance of predator/prey ratio does not fit with the Eltonian Pyramid of Shotwell (1) or the predator/prey ratio for endothermy in Bakker (5). Both of these authors stated that in a "normal" community, prey should make up approximately 90-98% of a fauna with predators comprising 2-10%. Poust stated that within the Brule Formation at Badlands National Park, prey animals comprised 90% of the paleofauna (6). Poust also stated that the entelodont, Archaeotherium, was not only a scavenger, but also a predator (6). Looking at the MNI's from the Pig Dig, the number of carnivores makes up 33% of the paleofauna. This percentage is much higher than would typically be expected (1, 5), and is significantly higher than the percentage of carnivores reported elsewhere from the Brule Formation (6). Also, the percentage of predators is much higher than what is seen in modern day Africa. A census of African animals focusing only on mammals, around water holes, indicates that the percentage of predators at the water holes is between 1-2% (7), which is significantly lower than the 33% at the Pig Dig Ouarry. Based on this analysis, a new interpretation of the Big Pig Dig as a modified "predator" trap is warranted. Carnivores became trapped in mud when trying to consume carrion, which may have been washed into a shallow basin as a result of a catastrophic event. This interpretation accounts for the high percentage of carnivores, which deviates from the typical 2-10%. The "green layer" could be interpreted as a muddy substrate the carnivores became mired in, and the green color could be attributed from the decomposition of the carrion creating a reducing environment.

Conclusions – On the basis of new information from MNI counts, the watering hole interpretation is not plausible and a new interpretation for the Big Pig Dig as a modified "predator" trap is proposed. This interpretation is supported by the high percentage of carnivores (33%), which is a large deviation from the expected 2-10% of a normal mammalian community. Finally, the "green layer" likely formed as a result of the carrion decomposing in a muddy substrate after being washed into shallow basin. Future work will include a closer examination of the geology and spatial orientation of the fossils, which may provide a more accurate taphonomic depiction of the site.

<sup>1)</sup>Shotwell A. (1955) Ecol. 36(2):327-337.

<sup>2)</sup>Stevens K. (1996) Unpublished Master's Thesis, South Dakota School of Mines & Technology, 103p.

<sup>3)</sup> Cavin J, Lien D, Herbel C, Johnson S, and Knauss G. (2002) Jour. of Vert. Pale., 22(3A):72.

<sup>4)</sup>Cavin J, Sheldon M, Weiler M, Johnson S, Tate A, and Herbel C. (2005) Jour. of Vert. Pale., 25(3A):43.

<sup>5)</sup>Bakker R. (1975) in, Ecological Studies, (Jacobs J, Lange O, Olson J, and Weiser W eds.), New York: Springer-Verlag, pp. 365-402.

 $<sup>6)</sup> Poust\ A.\ (2006)\ Abstracts\ with\ Programs-Geol.\ Soc.\ of\ Amer.,\ 38 (4):68.$ 

<sup>7)</sup> Weir J and Davison E. (1965) Zoologica Africana, 1(2):353-368.

### CAVEOLAR MICRODOMAINS AS ORGANIZERS OF CALCIUM SIGNALING

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Ca<sup>2+</sup> is a major signaling molecule in both excitable and non-excitable cells, where it modulates a variety of cellular functions ranging from cell growth to differentiation to cell death. Across a wide spatial and temporal range, several Ca<sup>2+</sup> channels generate Ca<sup>2+</sup> signals that can last from microseconds to minutes. This broad range of Ca<sup>2+</sup> signals can be efficiently coordinated through organization of specific Ca<sup>2+</sup> channels, pumps, buffers, exchangers and protein scaffolds at common microdomains. Lipid rafts/ caveolae serve as such a plasma membrane (PM) microdomain wherein highly specific signaling molecules are assembled so as to efficiently execute a defined signaling event with precision. Unlike the membrane rafts, which are planar, 'Caveolae' – a raft subset represent flask/omega shaped membrane invaginations that are widely expressed in a variety of cell types. Caveolae are cholesterol rich specialized PM domains with Caveolin1 (Cav1) as their major structural protein. These membrane domains represent a major cellular sub-compartment that facilitates various signal transduction events including Ca<sup>2+</sup> influx. Transient receptor potential canonical-1 (TRPC1) constitute an integral component of PM Ca<sup>2+</sup> channels that initiates store operated Ca<sup>2+</sup> entry (SOCE) following the depletion of endoplasmic reticulum (ER) Ca<sup>2+</sup>. One of the important questions regarding SOCE that has been actively pursued is about the molecular mechanism(s) whereby the status of the ER Ca<sup>2+</sup> store is communicated to the plasma membrane channels to initiate Ca<sup>2+</sup> influx. Recent, studies from several laboratories have identified the involvement of stromal interaction molecule-1(STIM1) in this process. STIM1 is expressed in the ER as a single transmembrane protein which can sense the changes in ER Ca<sup>2+</sup> levels. Following depletion of ER Ca<sup>2+</sup>, STIM1 forms clusters at the ER-PM junctional regions, where it physically associates with PM channels and activates SOCE. Here we demonstrate the association of TRPC1 and STIM1 with caveolar compartments of the PM. Depletion of ER Ca<sup>2+</sup> stores enhanced the recruitment of TRPC1 protein to plasma membrane rafts and its interaction with STIM1. Disruption of caveolar domains by sequestering membrane cholesterol altered raft-associated TRPC1 localization, its interaction with STIM1 and eventually SOCE. Additionally, silencing of Cav1 severely reduced the PM association of TRPC1 and in cav1 knockout tissues; membrane raft association of TRPC1 was abolished. Altogether, our findings demonstrate activation of TRPC1 relies on its association with STIM1 and on the integrity of caveolar micro-domains. In conclusion, we propose that the association of TRPC1 with Cav1 is required for targeting the channel to specific PM compartments, where it functionally associates with its activator STIM1, thereby, providing a substantial evidence for caveolae as organizers of TRPC1 mediated Ca<sup>2+</sup> signaling.

### **Funding:**

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# INCREASING THE SENSITIVITY OF ADVANCED STAGE OF PROSTATE CANCER TO CHEMOTHERAPEUTIC DRUGS-INDUCED APOPTOSIS BY TARGETING miR-205 AND miR-31

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PURPOSE: Prostate cancer is the major leading cause of death due to cancer amongst men in the United States. There are several chemotherapeutic drugs available for treatment if the cancer is diagnosed at an early (non-malignant) stage. However, there is no treatment available, till date, for the malignant form of cancer. Studies show that Prostate cancer is usually detected when it has reached the malignant stage (1). The purpose of this study is to identify the role of two microRNAs, *miR-205* and *miR-31*, in the regulation of apoptosis in two prostate cancer cell lines. WPE-1 NA22 represents the early stage of prostate cancer and WPE-1 NB26 represents the malignant form (2).

METHODOLOGY: We used microRNA expression analysis to find the microRNAs which are differentially expressed in the two cell lines and the Apoptotic assay to determine their response to various chemotherapeutic drugs. We also did Western blot analysis to see the difference in the protein expression of anti-apoptotic genes in these cell lines.

RESULTS: We have found that WPE-1 NB26 cells express less *miR-205* and *miR-31* than WPE-1 NA22 cells, and hence have higher levels of anti-apoptotic proteins like Bcl-w and E2F6, respectively. This makes them resistant to the treatment with various apoptotic agents. Also, the Bcl-w and E2F6 stable expressing WPE-1 NA22 cell lines showed high resistance to various chemotherapeutic drugs.

CONCLUSIONS: *miR-205* and *miR-31* play an important role in apoptosis induced by chemotherapeutic drugs and can be a useful strategy to treat the prostate cancer at an advanced stage.

- 1. Scardino P.T., et al, 1992, Human Pathology, 23, 211-222.
- 2. Webber M.M., et al, 2001, The Prostate, 47:1-13.

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PROFESSIONAL COMMUNICATIONS

## SCHEDULE OF PRESENTATIONS

Professional talks will be in the Peace Garden Room in the Memorial Union – session will be chaired by (to be determined)

# MORNING SESSION

- 7:30 Registration desk in the Legacy Lounge in the Memorial Union open
- 8:00 Greetings from President Prüß in the Century Theatre in the Memorial Union
- 8:20 LIFE HISTORIES AND BEHAVIORAL ECOLOGY OF AMPHIBIANS IN NORTHWESTERN NORTH DAKOTA. Christopher K. Beachy, Kenneth C. Cabarle, Kyna Hogg, and William Langer [p.84]
- 8:40 GENES ASSOCIATED WITH ENDOTHELIAL FUNCTION AND RISK OF PRE-ECLAMPSIA IN AN AMERICAN INDIAN POPULATION. Lyle G. Best, Melanie Nadeau, Shellee Bercier, Sara Dauphinais, Jacob Davis, Kylie Davis, Shyleen Poitra, Cindy M. Anderson. [p.77]
- 9:00 CHARACTERISTICS OF TWO MEASURES OF ENDOTHELIAL FUNCTION AMONG AMERICAN INDIAN PARTICIPANTS IN A CASE-CONTROL STUDY OF PRE-ECLAMPSIA. Lyle G. Best, Shellee Bercier, Jacob Davis, Kylie Davis, Shyleen Poitra, Cindy M. Anderson . [p.78]
- 9:20 IDENTIFICATION AND CHARACTERIZATION OF NOVEL MEMBRANE ASSOCIATED PROTEIN KINASES IN THE PATHOGEN *TRYPANOSOMA BRUCEI*. John A. Flaspohler, Bryan Jensen, and Marilyn Parsons. [p.79]
- 9:40 USING NEW, NOT SO OLD, AND TRADITIONAL GEOLOGICAL TECHNIQUESTO INTERPRET GEOLOGIC SECTIONS IN CRETACEOUS HELL CREEK FORMATION STRATA OF MONTANA. Matthew Burton-Kelly, Nels Peterson, Joseph H. Hartman, Gregory P. Wilson, Jeremy A. Riedel, and Allen Rice. [p.80]
- 10:00 INDOLE-3-ACETIC ACID-INDUCED LEAF GROWTH REQUIRES BOTH LEAF DETACHMENT AND SUBSTANTIAL WOUNDING. Morgan L. Grundstad, Michael Evanoff, Derek S. Lentz, Angela H. Culler, Jerry D. Cohen and Christopher P. Keller. [p.81]
- 10:20 BREAK (refreshments and exhibits in Plains Room in Memorial Union).
- 10:40 CHARACTERIZATION OF RABIES CASES IN ANIMALS IN THE MIDWESTERN UNITED STATES, 2000-2008. Susan Olet, Jennifer A. Tofteland, Neil Dyer and Margaret L. Khaitsa. [p.82]
- 11:00 HEAVY MINERAL ANALYSIS AND CORRELATION OF NORTH DAKOTA LATE EOCENE SANDSTONES. John R. Webster and Allen J. Kihm. [p.83]
- 11:20 PRODUCTION OF A HIS-TAG RECOMBINANT FAR PROTEIN AND ANALYSIS OF THE LIFECYCLE STAGES OF HP-FAR-1. Jennifer L. Bath Malcolm W. Kennedy, Jeremy Drees Erin Maetzold Michael Scheidt, Megan Knox, Daniel Ram, Peace Eneh, and Colin

**Clark**. [p.76]

- 11:40 GROUND-PENETRATING RADAR INVESTIGATION OF A RAPIDLY DEVELOPED ISLAND-LIKE FEATURE IN A SOUTH GEORGIA LAKE. Eric C. Brevik, Can Denizman, and Jim Doolittle [p. 85]
- 12:00 Lunch (served in the Plains Room in the Memorial Union). We will also conduct our business meeting (open to all members) during the lunch hour

# **EVENING**

6:00 Banquet will be in the Plains Room in the Memorial Union

# PRODUCTION OF A HIS-TAG RECOMBINANT FAR PROTEIN AND ANALYSIS OF THE LIFECYCLE STAGES OF HP-FAR-1

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Hp-FAR-1 is a major secreted antigen of the parasitic nematode *Heligmosomoides polygyrus*, a laboratory mouse model frequently used to study the cellular mechanisms of chronic helminth infections. The DNA encoding Hp-FAR-1 was previously discovered and isolated in our lab. In this abbreviated release of our findings, we provide the methods used to produce a functional his-tag Hp-FAR-1 protein. This method of purification will provides a simple and inexpensive protocol for use with multiple Hp-FAR-1 variants. In addition, we demonstrate stage specificity of Hp-FAR-1 transcription, demonstrating the stages of the parasites lifecycle against which host immunity to Hp-FAR-1 is likely to target. Retinol depletion as a result of Hp-FAR-1 binding activity is the first proposed mechanism to explain host immunosuppression that explains the cellular processes occurring during helminth infection.

The Hp-FAR-1 gene sequence was amplified for cloning purposes by PCR using primers designed to omit a putative signal peptide from the N-terminus of the protein. The PCR product was cloned directly into pET-30a+ and verified by sequencing. The plasmid was transformed into *E. coli* BL21 cells for expression of the recombinant protein. Hp-FAR-1 was purified on a nickel sepharose column and purity was confirmed by SDS-PAGE. The purified proteins were dialyzed at 4°C against PBS and passed through a column of Extracti-Gel D resin to remove any contaminating detergent.

*H. polygyrus* representing the infective stage, larval stage and adult stage were extracted as previously described (1). mRNA was extracted from each stage of the parasite and the gene encoding Hp-FAR-1 was amplified using Hp-FAR-1 specific primers and PCR.

The his-Hp-FAR-1 protein was successfully isolated, with the majority of the protein extracted in the soluble fraction and a single band present on SDS-PAGE. Ligand binding analyses showed that the Hp-FAR-1 bound the fluorophore-tagged fatty acid DAUDA, producing a significant blue shift in its peak emission, indicative of a highly apolar protein binding site. PCR amplification of the Hp-FAR-1 gene sequences verified the presence of a band at approximately 465 bp, corresponding to the size of the Hp-FAR-1 gene, by agarose gel electrophoresis. This band was present in all three stages of the lifecycle analyzed.

In this study we demonstrate an inexpensive and function-retaining method of Hp-FAR-1 protein production. It is of great interest to continue to explore the role that retinol and fatty acid depletion play in host immunity. Having a reliable, enzyme free method of protein production rovides us with an inexpensive way to produce Hp-FAR-1 and it's variants for pre-clinical and chemotherapeutic analysis in one of the most well-defined helminth mouse models. In addition, understanding the lifecycle production of Hp-FAR-1 will enhance the rational design of our treatments.

1) Robinson, M., T. Gustad, M. Erickson, J. Ferguson, and C. David (1997) Non-specific binding of IgG1 to *Heligmosomoides polygyrus*: adult worm homogenate superantigen is a target for immunoglobulin-induced inhibition Paras, Immunol.19: 469-474

# GENES ASSOCIATED WITH ENDOTHELIAL FUNCTION AND RISK OF PRE-ECLAMPSIA IN AN AMERICAN INDIAN POPULATION

Lyle G. Best, \*\* Melanie Nadeau<sup>1</sup>, Shellee Bercier<sup>1</sup>, Sara Dauphinais<sup>1</sup>, Jacob Davis<sup>1</sup>, Kylie Davis<sup>1</sup>, Shyleen Poitra<sup>1</sup>, Cindy M. Anderson, PhD<sup>2</sup>.

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The etiology of pre-eclampsia (PE) is unknown; but endothelial dysfunction has been hypothesized to play a role since at least the early 1990's (1). While a number of genes involved with endothelial function have been investigated, including the nitric oxide synthase gene (NOS3), dimethylarginine dimethylaminohydrolase (DDAHI), and G-protein  $\beta$  (GNB3), no susceptibility genes have been clearly identified to date (2).

Objectives: Since population specific prevalences of variants and background genetic effects may influence risk in different communities, our aims were to: 1) determine the prevalence of 5 single nucleotide polymorphisms (SNPs) of these three genes within this American Indian community; and 2) to investigate possible association of these variants with PE.

Methods: A case-control study has enrolled cases confirmed by chart review and controls, matched on date of the index infant's birth. Genotyping utilized a commercially developed, allele specific, real-time PCR method (Applied Biosystems "Tagman" assay).

Results: To date, 88 cases and 182 matched control genotypes have been obtained for at least one of the SNPs. Hardy-Weinberg equilibrium is satisfied for each of the 5 polymorphisms. Analysis of the rs233115 SNP in the 3' untranslated region (3' UTR) of *DDAH1* using McNemar Chi square comparisons of TT vs other genotypes, or CC vs other genotypes finds 70/35 (p=0.00091) and 12/22 (p=0.123) discordant pairs, respectively. Except for the rs233115 SNP, conditional logistic regression analysis using additive models failed to show association between genotype and PE, either in univariate or multivariate models adjusted for age, nulliparity, body mass index and smoking as covariates. For the rs233115 polymorphism, additive univariate and multivariate models as noted above show odds ratios of 1.5 (CI 1.02 – 2.24, p=0.0391) and 2.2 (CI 1.23 – 3.77, p=0.0074) respectively. The recessive model comparing the TT genotype with all others by conditional logistic regression gives univariate and multivariate odds ratios of 1.9 (CI 1.15-3.14, p=0.0113) and 1.9 (CI 2.85 – 5.69, p=0.0029) respectively. Using the Bonferroni correction for multiple testing (5 SNPs), would adjust the threshold p value considered statistically significant to p<0.01.

Conclusions: Akbar et al (3) have reported a haplotypic association between *DDAH1* SNPs (including a different one in the 3' UTR) and pre-eclampsia in a Finnish population. Although the significance of the derived p values are borderline, given correction for multiple testing; these data support the possibility of genetic influences on risk for PE mediated through the *DDAH1* gene.

Supported by NIH grant P20 RR016741 from the NCRR.

- 1 Roberts JM et al, Am J Hypertens. 1991; 4:700-8.
- 2 Chapell S et al, Clin Sci (Lond). 2006; 110:443-58.
- 3 Akbar F et al, Mol Hum Reprod. 2005; 11:73-77.

# CHARACTERISTICS OF TWO MEASURES OF ENDOTHELIAL FUNCTION AMONG AMERICAN INDIAN PARTICIPANTS IN A CASE-CONTROL STUDY OF PRE-ECLAMPSIA

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Background: The etiology of pre-eclampsia (PE) is unknown; but endothelial dysfunction has been hypothesized to play a role since at least the early 1990's. A hallmark of endothelial function is the release of vasodilatory substances (such as nitric oxide) in response to insufficient perfusion. This response can be monitored by tracking the increased pulse pressure after an ischemic stimulus.

Methods: A case-control study has enrolled cases confirmed by chart review and controls, matched on date of the index infant's birth. Measurement of endothelial function utilized the Endo PAT 2000 instrument (Itamar Medical Ltd, Caesarea, Israel), which calculates the ratio of pulse pressure increase before and after occlusion of circulation in the upper arm for 5 minutes (the reactive hyperemic index, RHI). The augmentation index (AI) is correlated with endothelial function;<sup>3</sup> and is another measure this instrument derives from the detected waveform.

Results: After initial training, repeated measurements were made on one subject, on 6 different days. Intrasubject RHI measures of this individual showed a mean of 1.32 with a standard deviation of 0.096. The standard deviation of repeated RHI values from a group of 40 children with type I diabetes was higher (0.261).<sup>4</sup> Table 1 shows the results of RHI and AI measures on 10 cases and 16 controls in this study.

Table 1.

	CASES		CONTROLS	p value*	
	MEAN	STD DEV	MEAN	STD DEV	
RHI	1.74	0.418	1.61	0.380	0.411
ΑI	29.4%	26.6%	19.7%	22.4%	0.327

<sup>\*</sup> Independent samples, t-test

Table 2 shows lack of correlation between these two measures of endothelial function; and between two potential covariates of RHI and AI.

Table 2.

	Pearson correlation	p value
RHI vs AI	-0.185	0.355
RHI vs age of participant	-0.112	0.577
RHI vs study temperature	0.203	0.311
AI vs age of participant	0.133	0.508
AI vs study temperature	-0.012	0.951

The standard deviation of RHI measurements in both cases and controls was higher (0.38) than previously reported among 42 women with and without PE (0.1).

Conclusions: In this small sample, neither RHI nor AI appeared to differ between cases with a history of PE and controls without previous PE. No correlations were detected between either of the two measures of endothelial function (RHI or AI); or in pair-wise comparison between either of these measures and age of participant or temperature of exam room.

Supported by NIH grant P20 RR016741 from the NCRR.

- 1 Roberts JM et al, Am J Hypertens. 1991; 4:700-8.
- 2 Quyyumi AA et al, J Clin Invest. 1995; 95:1747-55.
- 3 Soga J et al, Hypertens Res. 2008; 31:1293-8.
- 4 Haller MJ et al. Pediatric Diabetes. 2007: 8:193-8.
- 5 Yinon D et al, Eur Respir J. 2006; 27:328-333.

# IDENTIFICATION AND CHARACTERIZATION OF NOVEL MEMBRANE ASSOCIATED PROTEIN KINASES IN THE PATHOGEN TRYPANOSOMA BRUCEI

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Trypanosoma brucei, the causative agent of African sleeping sickness (African trypanosomiasais), is a single celled eukaryotic pathogen which uses the tsetse fly as its insect vector. The organism is responsible for significant morbidity and mortality in both human as well as domesticated animals (nagana) in widespread areas of Africa where the tsetse flies and the pathogen are endemic. Treatment options have historically involved toxic drug regimens that in many cases cause severe side effects. No vaccine is currently available due largely to the ability of the pathogen to undergo antigenic variation. The work to be presented represents basic research designed to identify potential drug therapy targets for African trypanosomiasis.

T. brucei has a complex life cycle involving dramatic environmental changes in its environment as it shift between the vertebrate bloodstream and the insect gut. The cellular structure and physiology are quite different in the two environments, suggesting that the organism must be able to sense and adapt to rapidly changing environmental conditions. In most organisms membrane-spanning signaling proteins play critical roles in extra- and intracellular sensing, thereby regulating a variety of cellular processes. Adaptation to environmental stressors such as nutrient concentration in most cases involve signal transduction mechanisms. The lack of characterized transmembrane signaling proteins in trypanosomatids led us to study several predicted transmembrane protein kinase encoding genes in T. brucei.

One putative protein kinase, Tb927.2.2720, is exclusively expressed in bloodstream form parasites. This protein specifically localizes to the flagellar pocket, thought to be the sole site of cell sensory functions as well as endo- and exocytosis in this stage of parasite growth.

Another putative protein kinase, Tb11.01.0670, is expressed at the mRNA level in both procyclic (insect form) and bloodstream forms and encodes a protein (Tb670) that localizes to the monolayer lipid membrane of lipid body organelles in both procyclic and bloodstream stage parasites. RNAi studies in bloodstream forms demonstrated the importance of Tb670, as RNA knockdown led to growth arrest, which was accompanied by the disappearance of lipid bodies. Cell culture conditions which normally induce lipid body formation in procyclic cells failed to act as potent lipid body inducers in Tb670 knockdown cells. While immunoprecipitated Tb670 protein possesses protein kinase activity, the activity under normal culture conditions is modest. Hence we propose that Tb670 kinase activity is regulated by nutrient status, and that it is involved in lipid body biogenesis and/or the mobilization of lipids in response to changing environmental stimuli. Through the studies described we hope to gain a foothold in understanding the basic components required for cell signaling in these important pathogens.

# USING NEW, NOT SO OLD, AND TRADITIONAL GEOLOGICAL TECHNIQUESTO INTERPRET GEOLOGIC SECTIONS IN CRETACEOUS HELL CREEK FORMATION STRATA OF MONTANA

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**INTRODUCTION.** New and updated techniques are applied to data gathering every day with rapidly changing technology. During the 2008 field season, a team of paleontologists and geologists studying the uppermost Cretaceous Hell Creek Formation in its type area in Garfield County, Montana, had the good fortune to have Flag Butte scanned with Riegl Z390 LiDAR (light detection and ranging) by Peterson and Rice. The objective of the scan was precise placement of observations (made by various means) on this important butte. This report is an initial testament to our effectiveness in correlating GPS (global positional system) data to a LiDAR surface.

METHODS-LiDAR. In badlands terrain, this involved completing a sight survey, followed by the placement of a collection of retro-reflective markers throughout and on the perimeter of the study area. Each reflector is required to be in line-of-sight from several locations. After the reflectors were placed, the Riegel was placed at a location and a scan made to get the precise location of each of the retro-reflective markers. After initializing the retro-reflective markers, a surface scan of point data (in all directions) was made of the area at a resolution of at least 0.1 degree. After scanning was completed at all positions, each scan position was searched and its respective reflector located. This location information was translated into a project coordinate system. Once all of the scans were so linked together, we were able to take WAAS-optimized GPS data from a Garmin Legend® GPS for several of the retro-reflective reference points that we collected and referenced the project coordinate system to a global coordinate system, which, in this case was a UTM coordinate system with a WGS87 datum. Peterson subsequently created the LiDAR surface (at the Museum of the Rockies, with the support of the Museum of the Rockies through the assistance of John Horner). Digital photographs taken from the LiDAR stations were superimposed with the LiDAR surface.

METHODS-GPS AND GIS. Hartman and Burton-Kelly acquired the LiDAR data from Peterson and, with the generous assistance of Sue Martin, Operations Manager/Controller of Riegl USA, Inc., were able to process the data at the University of North Dakota. As part of our learning experience, likely not the most elegant method was chosen in ESRI ArcGIS 9.2 (geographic information systems) to determine the relative elevation difference between the LiDAR surface and our secondary GPS waypoints. Note that LiDAR station control points were located with the Garmin Legend®, but LiDAR surface elevation control was configured with the aid of higher-resolution GPS data collected by Jeremy Riedel and Greg Wilson using a Trimble GeoXT<sup>TM</sup>. These data were taken during a paleomagnetic survey under the supervision of William C. Clyde and Rebecca M. LeCain (University of New Hampshire). GPS waypoints of measured sections and fossil localities were taken by Hartman and Arthur E. Bogan (North Carolina Museum of Natural Sciences) using WAAS-optimized GPS units, including a Garmin GPSmap76 and DeLorme Earthmate® GPS PN-20

Of the 16 GPS section values measured, most of which were taken on the perimeter of the LiDAR scan, the average Earthmate® GPS elevation error recorded in November 2008, was 4.29 m, with a range of 2.13 to 7.01 m. Almost all of the actual GPS readings occur above the scanned LiDAR surface by an average of 9.55 m, ranging in value from -0.69 m (one reading below) to 26.54 m. The four GPSmap76 fossil locality waypoints taken in July 2008 average 7.01 m above the LiDAR surface, with a tight range of 6.65 to 7.64 m (no GPS field measurement error was recorded). Additional Earthmate® GPS observational waypoints were taken within the scanned area in July. These elevations average 3.90 m above the LiDAR surface, ranging from 0.02 to 8.44 m. The GPS receiver error average for these reading was 4.75 m, ranging from 3.05 to 6.40 m.

**SUMMARY.** The LiDAR method holds great promise to establish a surface "ground" truth for field observations. The current project highlights sources of error that need better control to optimize interpretation of "corrections" to be precisely applied to geological and paleontological elevation field data. The general availability of LiDAR scans will provide a means to precisely transfer low-tech and inexpensively derived field data to all interested parties without loss of fidelity.

# INDOLE-3-ACETIC ACID-INDUCED LEAF GROWTH REQUIRES BOTH LEAF DETACHMENT AND SUBSTANTIAL WOUNDING

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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 <u>intact</u> expanding leaves of *Arabidopsis* and *Phaseolus*, either through exogenous application or through trapping the endogenous hormone in leaves, results in inhibition of leaf growth. Paridoxically, other work has clearly shown that treatment of excised leaf strips from tobacco (*Nicotiana*) with IAA stimulates rather than inhibits growth. 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 project used the model plant Arabidopsis to ask 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 (possibly involving a wound-induced collapse in endogenous hormone level as has been suggested<sup>3</sup>) or is the result of detachment from the plant and presumed separation from other growth controllers.

For our experiments, *Arabidopsis* seedlings were grown in moist potting soil in a growth chamber at 19°C, with continuous illumination (150 µM s<sup>-1</sup> m<sup>-2</sup>). After 10-14 days, plants were selected with both the first two true leaves 2.7-3.3 mm in diameter and rapidly expanding. 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. For most experiments, scaled digital images of the individual leaves were prepared for subsequent determination of initial leaf area; for others initial images of excised leaf strips (0.7 mm wide cut transversely across the midpoint of the leaves) were prepared. Either leaf strips or intact attached, detached, or wounded (sliced transversely from leaf edge to near the midvein in three places) attached leaves were treated 24 hours with full strength Murashige and Skoog media (with 10 mM KCl and 0.1 mM Mes/Btp (pH 6.0)) or the same +/- IAA at various concentrations. After 24 hours increased area and epinatic curvature of leaves and strips was determined from digital images. The effect of wounding and of leaf detachment on endogenous leaf IAA content was determined using a new high-sensitivity high-throughput assay<sup>4</sup>.

The growth of intact attached leaves was found to be somewhat insensitive to IAA. While lower concentrations of IAA were ineffective higher concentrations were inhibitory. Wounded-attached leaves showed a similar response to exogenous IAA treatment. The growth of detached leaves was also inhibited by IAA but was more sensitive, although this sensitivity was lost at higher light intensities. Only excised leaf strips were induced to grow more rapidly when treated with IAA. Epinastic curvature was strongly induced in all experiments regardless of leaf treatment. Wounding and leaf detachment was found to have no significant effect on the endogenous leaf IAA level which was found to be lower than the effective exogenous concentrations.

The results suggest that IAA-induced growth increase results from a wound response that requires substantial wounding (simple leaf excision is not enough) as well as excision for the plant.

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

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<sup>&</sup>lt;sup>2</sup> Keller CP, Van Volkenburgh E (1997) Plant Physiology 113: 603-610.

<sup>&</sup>lt;sup>3</sup>Thornburg RW, Li X (1991) Plant Physiol. 96:802-805

<sup>&</sup>lt;sup>4</sup>Barkawi et al. (2008) Analytical Biochem. 372: 177-188

# CHARACTERIZATION OF RABIES CASES IN ANIMALS IN THE MIDWESTERN UNITED STATES, 2000-2008.

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Rabies is a zoonosis that inflicts nearly all mammalian species worldwide, including humans, skunks, raccoons, dogs, cats, foxes, and bats (1). The risk associated with rabies among wild animals species varies from one country to another. In the US the greatest risk of rabies is attributed to bats, skunks and raccoons. Although no deaths among humans have been documented during the period 2000-2008 in North Dakota (ND) the risk associated with wild animal rabies and the reintroduction of canine rabies in the United States (US) exists (2).

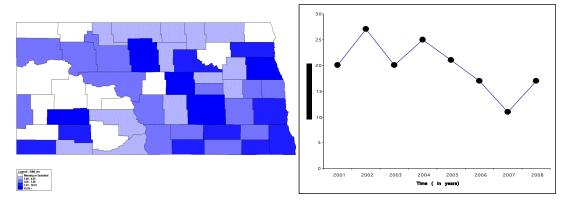
This report summarizes data from North Dakota Veterinary Diagnostic Laboratory (NDVDL) for the period 2000 to 2008 on rabies epidemiological patterns by year, season, species, state and county. Data were analyzed using SAS version 9.13 and EPIINFO windows version 3.3. Graphs and maps were used to show trends and spatial distribution of rabies cases reported. The effect of time (in years) on the number of rabies cases reported in each county over the 8 years period (2001-2008) was investigated using a repeated measures analysis assuming a Poisson distribution.

Preliminary results indicate that over the study period, a total of 252 animals tested positive for rabies with the majority (78.2%, 197/252) reported in skunks and (21.8%, 55/252) in other species including bovine (8.3%, 21/252), canine (2.8 %, 7/252), feline (4.0%, 10/252), horses (2.4%, 6/252), bat (2.4%, 6/252), badger (0.4%, 1/252), donkey (0.4%, 1/252), raccoon (0.8%, 2/313), and goat (0.4%, 0.4%/313). Most counties (90.6%, 48/53) reported at least one case of rabies in animals with counties in Southeastern ND reporting most cases (Figure 1). On average the number of rabies cases significantly decreased over the years (p = 0.0032) (Figure 2). Additional results on further analysis are pending.

There appears to be a bi-annual cyclic pattern in the counties reporting 4-7 cases and in the total number of cases reported annually over the years possibly due to fluctuation of populations at risk. A seasonal pattern was observed regarding the mean monthly cases of rabies reported across the study years with the mean number of cases peaking during the months of April to July. With the skunk as the major terrestrial animal reservoir in ND, rabies control strategies could target this species. Also, control strategies could be applied biannually given the apparent cyclic nature of the disease in ND animals; and across the whole state given the wide distribution of cases

Figure 1: Distribution of all rabies cases reported in North Dakota by county (2001-2007)

Figure 2: Distribution of rabies cases in North Dakota by year (2001-2008)



1. Sun, B., et al. (2008). Compendium of animal rabies prevention and control. MMWR Recommendations and Reports. 57(RR02);1-9

<sup>2.</sup> Briggs, D. et al. (2004). WHO expert consultation on rabies. WHO Technical Report Series. First Report.

# HEAVY MINERAL ANALYSIS AND CORRELATION OF NORTH DAKOTA LATE EOCENE SANDSTONES

# John R. Webster\* and Allen J. Kihm Minot State University, Minot, ND 58707

Late Eocene sandstone exposed at the Medicine Pole Hills (MPH) in southwestern North Dakota (11 km south of Rhame) is a conglomeratic arkose that unconformably overlies the Paleocene Tongue River Formation (Fort Union Group). The MPH sandstone has been correlated by Murphy et al. (1) with the conglomeratic arkosic sandstone of the Chalky Buttes Member (CBM) of the Chadron Formation (White River Group), which is exposed at numerous buttes in the North Dakota-South Dakota-Montana tri-state area (2). The MPH sandstone has produced abundant vertebrate fossils (MPH Local Fauna) that indicate an early Chadronian age (3). Correlation of the MPH sandstone with the CBM is significant because it would constrain the age of the CBM, which has produced relatively few fossils (1). Heavy mineral analysis is being used to test the correlation of these sandstones.

The MPH sandstone was sampled from a pit excavated at the top of one of the hills. The CBM sandstone was sampled at several buttes; thus far, samples from Rattlesnake Butte (RSB) and Square Butte (SQB) have been studied. The samples (approximately 4 kg) were disaggregated, cleaned to remove silt and clay, and separated into ¼-φ grain size fractions. Heavy minerals were separated from fine to medium-grained sand (0.25-0.30 mm; also 0.212-0.25 for RSB) using a heteropolytungstate solution (2.85 g/cm³). Polished grain mounts were prepared on one-inch round glass slides. Heavy mineral grains (400-600 per sample) were identified using optical microscopy, and for some grains microanalysis with a scanning electron microscope-energy dispersive spectrometer system.

Samples studied thus far consisted of medium-grained, well- (MPH) to moderately sorted (RSB and SQB) sand. The overall abundance of heavy minerals in the separated grain size(s) varied considerably (Table 1) as did the percentages of heavy minerals that were opaque (0.43% in MPH, 8.52% in RSB, and 16.51% in SQB). Non-opaque heavy mineral abundances (Table 1) were also very different in the three samples. The MPH sample was dominated by diopside and hornblende; RSB by epidote, garnet, and biotite; and SQB by rutile, epidote, staurolite, and apatite.

Table 1. Results of heavy mineral analyses

				Normalized Percentages of Non-opaque Minerals										
	grain size (mm)	% heavy minerals	horn- blende	diopside	biotite	garnet	epidote	alumino- silicate	staurolite	apatite	zircon	tour- maline	rutile	other
MPH	0.25-0.30	8.5	26.1	61.6		3.3	6.9							2.1
RSB	0.212-0.30	0.35	0.3		24.2	24.3	47.1	0.7	0.3		0.1	0.6	0.7	1.7
SQB	0.25-0.30	0.07	1.6		0.3	3.4	13.5	4.7	10.7	9.7	1.3	3.4	48.3	3.1
CF <sup>a</sup>	0.063-0.125		3		2	7	10	14	22		28	10	3	1

<sup>&</sup>lt;sup>a</sup> average of Chadron Formation samples of Denson et al. (2); predominantly sampled from the CBM

The distinct heavy mineral assemblages in the MPH, RSB, and SQB are interpreted to represent rather different sources. MPH was dominated by a Tertiary volcanic (Tv) source, with some Precambrian metamorphic-plutonic (PCmp) source input. RSB was dominated by a PCmp source, with some input from Tv. SQB was dominated by ultrastable grains from recycled sediment (RS) and/or a PCmp source, and other grains from a PCmp source. The MPH and RSB samples had similar PCmp sources, but very different Tv sources. The SQB source(s) (RS and/or PCmp) were very different from MPH and RSB sources. All three samples differed from the average Chadron Formation heavy mineral analysis (Table 1) reported by Denson et al. (2). Overall, the distinct heavy mineral assemblage in the MPH sandstone suggests it should not be correlated with the CBM sandstone. Additionally, the CBM sample results (and Chadron Formation data) suggest the CBM sandstone may represent a complex depositional setting that consisted of multiple depositional systems receiving sediment from multiple, changing sources through a significant interval of time.

<sup>1)</sup> Murphy EC, Hoganson JW, and Forsman NF. (1993) ND Geologic Survey Report of Investigation 96, 144 p.

<sup>2)</sup> Denson NM, Gill JR, and Chisholm WA. (1965) USGS Prof. Paper 463, 75 p.

<sup>3)</sup> Heaton TH, and Emry RJ. (1996). *in*, The terrestrial Eocene Oligocene transition in North America (Prothero DR and Emry RJ eds.), New York: Cambridge University Press, pp. 581-608.

# LIFE HISTORIES AND BEHAVIORAL ECOLOGY OF AMPHIBIANS IN NORTHWESTERN NORTH DAKOTA

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In 2005, the Amphibian Growth Project established drift fences surrounding two temporary ponds in Ward and McHenry Counties, North Dakota. A third drift fence was added at Wolford, North Dakota in 2007. These sites have produced approximately 4000 amphibian captures of six different species: one salamander (Ambystoma tigrinum) and five frogs (Rana sylvatica, Rana pipiens, Bufo cognatus, Pseudacris maculata, and Scaphiopus bombifrons). In addition, in 2008 we established five additional localities across northern North Dakota where annual seasonal samples are collected. All specimens are assayed for size (SVL and mass). In addition, some specimens can be externally sexed. Several destructive samples have been taken for all species to assess reproductive condition. Finally, many specimens that are large enough have been injected with a passive integrated transponder tag in order to identify individuals. observations have generated a large database that we are using to develop estimators of migration behavior, growth rates, fecundity, mortality rates, and population sizes. We are using these estimators to elucidate the local life histories of these species in agricultural settings. This research was supported by NIH Grant Number P20 RR016741 from the INBRE Program of the National Center of Research Resources and by a State Wildlife Grant from the North Dakota Department of Game and Fish.

# GROUND-PENETRATING RADAR INVESTIGATION OF A RAPIDLY DEVELOPED ISLAND-LIKE FEATURE IN A SOUTH GEORGIA LAKE

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On the morning of October 13, 2006, the residents of a small private lake in south Georgia woke up to discover a new addition to their lake: a small "island" that had never before existed (Figure 1). This particular region of south Georgia is characterized by karst developed in a relatively stable tectonic setting covered by either thick impermeable siliciclastics or thin layers of Pleistocene deposits. Sinkholes (dolines) are the ubiquitous landform in the temperate karst of southern Georgia. Because south Georgia is in an active karst zone, a karst explanation was deemed most likely in this case. Past successes using ground-penetrating radar (GPR) to investigate karst features in the southeastern United States and in freshwater bodies lead to the use of GPR as the primary means of investigating this particular phenomenon.

The radar unit used was the TerraSIRch Subsurface Interface Radar (SIR) System-3000 ®, manufactured by Geophysical Survey Systems, Inc. (Salem, NH). A 70 MHz antenna was used in this survey. This antenna provided adequate depth (greater than 10 m) and acceptable lateral resolution of subsurface features. Radar records were processed with the RADAN for Windows ® (version 5.0) software program. An Allegro CE ® field computer (Juniper Systems, North Logan, Utah) and a Garmin Global Positioning System Map 76 ® receiver (Garmin International, Inc., Olathe, Kansas) were used to record the coordinates of each reference station that was impressed on the radar record. SURFER for Windows ® (version 8.0) (Golden Software, Inc., Golden, CO) was used to construct images of the estimated depths to bottom sediments using kriging methods. The radar system was mounted in a fiberglass boat towed behind a pontoon boat (Figure 1).

Radar records were of excellent interpretative quality. Figure 2 is a portion of a representative radar record. This portion of the radar record crossed the impacted area in an east-northeast to west-southwest direction. In Figure 2, the emergent "island" is most closely approached between reference points 5 and 6. The high-amplitude reflections at "A" represent the lake bottom, revealing a deep crater-like feature and up-thrust ridges at the eastern side of the crater similar to the "island" seen above water level. The high-amplitude planar reflector at "B" likely represents the upper boundary of the underlying limestone bedrock.

Results indicate that the "island" seen above water was formed as a thrust ridge along the eastern edge of a sinkhole that opened in the lake late on October 12 or early on October 13, 2006. The thrust ridge is composed of lake sediments that were probably pushed up along a hinge-line created by the rotational collapse of an intact slab of limestone and/or pore water pressure created by the collapse.

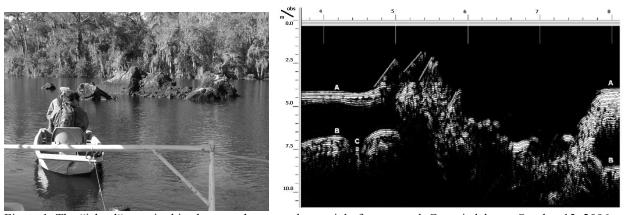


Figure 1. The "island" seen in this photograph emerged overnight from a south Georgia lake on October 13, 2006. Figure 2. A deep sinkhole and up-thrust ridges of sediment are evident in this portion of the GPR record.

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# CONSTITUTION of the NORTH DAKOTA ACADEMY OF SCIENCE

Founded 1908, Official State Academy 1958

# ARTICLE I - Name and Purpose

Section 1. This association shall be called the NORTH DAKOTA ACADEMY OF SCIENCE.

Section 2. The purpose of this association shall be to promote and conduct scientific research and to diffuse scientific knowledge.

## ARTICLE II - Membership

Membership in the Academy shall be composed of persons who share the stated purpose of the Academy and who are active or interested in some field of scientific endeavor.

#### ARTICLE III - Council

The officers of the Academy shall be a President, a President-Elect, and a Secretary-Treasurer. The Council, consisting of the officers, the retiring President, and three elected Councilors, shall be responsible for the fulfillment of the scientific and business obligations of the Academy.

# ARTICLE V - Dissolution and Limits of Action

Section 1. In the event of dissolution of the Academy, any remaining assets shall be distributed to organizations organized and operated exclusively for education and scientific purposes as shall at the time qualify as exempt organizations under Section 501(c) (3) of the Internal Revenue Code of 1954.

Section 2. No substantial part of the activities of the Academy shall be the carrying on of propaganda, or otherwise attempting to influence legislation, and the Academy shall not participate in or intervene in, any political campaign on behalf of any candidate for public office.

Section 3. No part of any net earnings shall inure to the benefit of, or be distributable to, Academy members or officers, or other private persons, except that the Academy may authorize the payment of reasonable compensation for services rendered.

# ARTICLE VI - Amendments

Section 1. This Constitution may be amended at any annual Business Meeting of the Academy by a two-thirds vote. Proposed amendments shall be submitted in writing to the Secretary-Treasurer who shall send them to the members at least two weeks before the meeting at which such amendments are to be considered.

Section 2. Bylaws may be adopted or repealed at any regular business meeting by a two-thirds vote.

# **BYLAWS**

# BYLAW 1. Meetings

Section 1. Scientific Meetings. The Academy shall hold at least one annual scientific meeting each year at a time and place determined by the Council. Other scientific meetings, regional, state, or local, may be held at times and places determined by the Council. The Council shall establish regulations governing the presentation of papers at Academy sessions. Such regulations shall be made available to members at least three months before any meeting at which they are to apply.

Section 2. Business Meetings. A Business Meeting of the membership shall be scheduled at the regular, annual scientific meeting of the Academy. Ten percent of the active members shall constitute a quorum at the annual

business meeting.

- Section 3. Special Meetings. Special meetings shall be called by the President upon the request of ten percent of the active members and require twenty percent of the active members for a quorum. Notice of the time and place of such meetings shall be sent to all members of the Academy at least four weeks in advance of the meeting. Only matters specified in the call can be transacted at a special meeting.
- Section 4. *Procedure*. Parliamentary procedures to be followed in all business meetings shall be those specified in "Standard Code of Parliamentary Procedure" by Alice F. Sturgis.

#### BYLAW 2. Financial

- Section 1. Fiscal year. The fiscal year shall run concurrently with the calendar year from January 1 to December 31.
- Section 2. *Dues and Assessments*. The annual dues and assessments may be changed from time to time by the Council, subject to approval by a two-thirds vote of the members at an annual Business Meeting. These dues are payable by January 31 for the current fiscal year or by the Annual North Dakota Academy of Science Meeting for those registering for the meeting
- Section 3. *Supporting Members*. Council shall maintain a program to encourage members to voluntarily contribute funds over and above the regular dues and assessments for the support of activities of the Society.
- Section 4. *Sustaining Members*. Any association, corporation, institution, or individual desiring to support the Society with funds or services valued at \$50 or greater may be invited by the President or designee to become a Sustaining Associate.
- Section 5. Audit and Reports. The Nominating Committee shall appoint on a yearly basis one member who is not a member of Council to conduct at least one internal audit per year. The Secretary-Treasurer shall report on the financial affairs of the Society, including the results of an annual audit, as may be requested by the Council.

# BYLAW 3. Membership

- Section 1. *Membership Categories*. Classes of membership shall include the following: (a) Regular, (b) Student, (c) Emeritus, (d) Honorary, (e) Supporting, (f) Sustaining, and (g) Lifetime Members.
- Section 2. *Eligibility and Procedure for Membership*. Candidates for membership, except Sustaining Member, may be proposed by any regular or emeritus member of the Academy by submitting the candidate's name to the chairman of the Membership Committee.
- (a) *Regular Members*. Any person who is active or interested in some field of scientific endeavor shall be eligible for regular membership. A majority vote of Council shall elect to regular membership.
- (b) *Student Members*. Any student who is an undergraduate or graduate student in some field of science shall be eligible for student membership. A majority vote of Council shall elect to regular membership.
- (c) *Emeritus Members*. Any member in good standing upon formal retirement is eligible for emeritus membership. A majority vote of Council shall elect to emeritus membership.
- (d) *Honorary Members*. The Academy may recognize, by awarding honorary membership, any person (nonmember or member) who has in any way made an outstanding contribution to science. It shall be the responsibility of the Membership Committee to be aware of individuals whom it would be fitting for the Academy to honor in this fashion. A two-thirds vote of members attending the annual business meeting shall elect to honorary membership.
- (e) Supporting Members. Regular or student members may voluntarily contribute funds over and above the regular dues and assessments for the support of activities of the Society.
- (f) Sustaining Associates. Any association, corporation, institution, or individual desiring to support the Society with funds or services valued at \$50 or greater may be invited by the President or designee to become a Sustaining Associate.
  - (g) Lifetime Members. Any regular member in current good standing for at least one year may become a

Lifetime Member by paying an assessment equal to 18 times the current annual dues in one lump sum or in two equal payments over the current and following year.

# Section 3. Privileges of Membership.

- (a) Voting at the annual business meeting is permitted of regular and emeritus members.
- (b) Members of all categories may attend business meetings of the Academy.
- (c) The Secretary-Treasurer and members of Council must be regular members in good standing.
- (d) Regular, student, and emeritus members may submit abstracts or communications for scientific meetings of the Academy.
  - (e) Emeritus and Honorary Members shall be exempt from payment of dues.
- (f) A Sustaining Member is provided a display area at the annual scientific meeting of five linear feet per \$50 donation up to a maximum of 20 linear feet.
- (g) Every member in good standing shall receive a printed copy or an electronic copy (if available and of equal or lesser cost than the printed copy) of the annual *Proceedings of the North Dakota Academy of Science*, the form to be determined by the member.
- (h) Special offices such as Historian may be created by the unanimous vote of the regular members at the annual Business Meeting.
  - (i) All student research participants shall receive a properly inscribed certificate.

# Section 4. Forfeiture of Membership.

- (a) *Nonpayment of dues*. Members shall be dropped from the active list on 31 November following the nonpayment of dues during the membership year commencing the previous 1 December. A member may return to the active list by paying the current year dues.
- (b) Expulsion for Cause. Membership may be terminated for conduct injurious to the Academy or contrary to the best interests of the Academy. The accused member shall be given an opportunity for a hearing before the Council. If a majority of the Council votes to expel the member, the action must be ratified by at least two-thirds of the members present at the next annual business meeting of the Academy. An expelled member shall forfeit all paid dues and assessments.

# BYLAW 4. Duties and Responsibilities of the Council and Council Members

- Section 1. Council. The Council shall meet, at the call of the President, at least twice a year. The Council shall:
  - (a) be the governing board of the Academy, responsible only to the membership.
- (b) arrange for programs, approve committee appointments, be responsible for the fiscal affairs of the Academy, and transact such business as necessary and desirable for function and growth of the Academy.
  - (c) determine the location of the annual meeting three years in advance.
- (d) annually appoint an Academy representative to the National Association of Academies of Science and to Section X (General) of the American Association for the Advancement of Science.
  - (e) shall appoint and may compensate a Secretary-Treasurer.
  - (f) shall appoint and may compensate an Editor of the PROCEEDINGS and other publications.
  - (g) shall be empowered to charge a publication fee of authors on a per page basis.
  - (h) shall control all activities of the Academy including grant applications.
- Section 2. *President*. The President shall preside at meetings of the Council and over the annual business meeting of the Academy at the close of the regular term office. The President shall vote only to break a tie. Unless otherwise specified, the President shall, with the approval of the Council, appoint members to serve on Standing Committees and *ad hoc* Committees, designate the chair of each Committee, and appoint representatives to other organizations. The President serves as Coordinator of the Local Arrangements Committee for the annual meeting that occurs at the end of the President's term.
- Section 3. *President-Elect*. The President-elect shall be considered a vice president and shall serve as such in the absence of the President.
- Section 4. *Past-President*. The retiring President shall serve as Past-President and chair of the Nominating Committee. The Past President shall serve ex officio on those committees designated by the President and shall serve in the absence of the President and President-elect.

- Section 5. Secretary-Treasurer. The Secretary-Treasurer shall:
  - (1) Assist Council in carrying on the functions of the Academy including the receipt and disbursement of funds under the direction of Council.
  - (2) Manage the Academy Offices under Council's general supervision.
  - (3) Serve as Managing Editor of the *Proceedings of the North Dakota Academy of Science*.
  - (4) Prepare a summary of the most recent audit and a report of the Academy's current financial status. This information shall be shared with the membership at the annual business meeting and published in the PROCEEDINGS following the business meeting.
  - (5) Perform all other duties of the Secretary-Treasurer listed in the Bylaws.
- (6) Serve as archivist and be responsible for all official records, archives, and historic material which shall be in reposit with the Secretary-Treasurer.

# BYLAW 5. Appointment, Nomination and Election of Members of Council

- Section 1. *Eligibility for Office*. All candidates for election or appointment to the Council must be regular members in good standing. Nominees for President-elect must be members who reside within easy commuting distance of the site of the annual meeting selected by the Council that occurs when the President-elect serves as President.
- Section 2. *Nomination Procedures*. The Nominating Committee shall be responsible for all nominations to elective office, shall determine the eligibility of nominees, shall ascertain that nominees are willing to stand for office, and shall be required to advance to the Secretary-Treasurer at least two names for each open position as needed. Academy members shall have been encouraged to suggest nominees to the committee prior to the Committee submitting its report.
- Section 3. *Election Procedures*. Election shall be by secret mail ballot. The Secretary-Treasurer shall prepare a printed ballot that bears all names submitted by the Nominating Committee, that contains a brief biography of each candidate, and that has space for write-in candidates for each office. This ballot is to be mailed to all members no later than 1 November. Each member wishing to vote must return the marked ballot in a sealed signed envelope to the Secretary-Treasurer postmarked not more than thirty days after the ballots were mailed out to members. The President shall appoint tellers, who shall count the ballots that have been received by the Secretary-Treasurer and the tellers shall present the results in writing to the President. A plurality of the votes cast shall be necessary to elect and in the case of a tie vote, the President shall cast the deciding vote. The results of the election shall be announced at the annual Business Meeting.
- Section 4. *Term office*. A President-Elect shall be elected annually by the membership and the following years shall succeed automatically to President and Past President to constitute a three-year nonrenewable term. Three Councilors shall be elected by the membership to three-year, non-renewable terms on a rotating basis. All elected Council members shall take office at the end of the next annual Business Meeting following election and shall continue until relieved by their successors. Council is empowered to appoint and compensate a Secretary-Treasurer to successive three-year terms that commence with the beginning of the fiscal year.
- Section 5. Removal from office or position. If for any reason any elected member of Council is unable to fulfill his/her duties, the Council member may be removed from office by two-thirds vote of Council. If for any reason the Secretary-Treasurer is unable to fulfill his/her duties, the Secretary-Treasurer may be relieved of all duties by a majority vote of Council.
- Section 6. *Interim vacancies*. Should a vacancy occur in the Presidency, the Council by a majority vote shall appoint a member of the Academy able to coordinate the next annual meeting to fill the unexpired term. A retiring interim President shall succeed automatically to Past President. Should a vacancy occur in the Presidency-elect, the Council shall reassess and change the location of the coinciding annual meeting as necessary and then call for a special election by mail ballot. An interim vacancy in the Past-Presidency shall be filled by the most recently retired Past-President able to fill the duties of the Past-President. Persons appointed to fill the unexpired term of Secretary-Treasurer are expected to remain in the position for a minimum of three years. A vacancy in the office of Councilor shall be filled by a majority vote of Council until the following election at which time the interim Councilor may stand for a full three year nonrenewable term.

## BYLAW 6. Committees

- Section 1. *Standing Committees*. Standing committees shall include but not be limited to, the following: Editorial, Education, Denison Award, Necrology, Nominating, Resolution, Membership, and Audit Committees. The President shall appoint members of committees other than the Nominating and Audit Committees.
- Section 2. *Editorial Committee*. The Editorial Committee shall consist of three regular members appointed to three year terms. The duties are explained in BYLAW 7 (Publications).
- Section 3. *Education Committee*. The Education Committee shall consist of five regular members and two high school teachers appointed to five year terms. The Education Committee shall work with high school students and teachers in the state, in visitation programs, Science Talent Search programs, and other programs to stimulate an interest in science by the youth of the state. It shall operate the Junior Academy of Science program and administer the AAAS high school research program.
- Section 4. *Denison Awards Committee*. The Denison Awards Committee shall consist of six regular members appointed to three year terms. The Denison Awards Committee shall have as its prime duty the judging of student research and paper competitions, both undergraduate and graduate, and any other similar competitions. The committee shall also maintain the criteria to be used in the judging and selection of papers, such criteria to be circulated to prospective competitors.
- Section 5. Necrology Committee. The Necrology Committee shall consist of three regular members appointed to three year terms. The Necrology Committee shall report to the annual meeting on those deceased during the preceding year. Obituaries may be included in the minutes of the annual meeting and/or published in the Proceedings.
- Section 6. Nominating Committee. The Nominating Committee shall consist of the five most recent past-presidents. The major duties of the Nominating Committee are listed in BYLAW 5 (Appointment, Nomination and Election of Members of Council). The Nominating Committee will also administer the selection process, develop a separate funding source for a monetary award, and develop, for Executive Committee approval, the criteria for the North Dakota Academy of Science Achievement Award.
- Section 7. *Resolution Committee*. The Resolution Committee shall consist of three regular members appointed to three year terms. The Resolution Committee shall prepare such resolutions of recognition and thanks as appropriate for the annual meeting. Further, the Committee shall receive suggested resolutions for the membership and transmit such resolutions and the Committee recommendation to the membership.
- Section 8. *Membership Committee*. The Membership Committee shall consist of unlimited numbers of regular members appointed annually.
- Section 9. *Audit Committee*. The Nominating Committee shall appoint on a yearly basis one member who is not a member of Council to conduct at least one internal audit per year.
- Section 10. State Science Advisory Committee. The State Science Advisory Committee (SSAC) shall consist of five regular or emeritus members appointed to four year terms. The SSAC shall serve to direct questions of a scientific nature to the appropriate expert as requested, shall inform regional granting agencies and state and national science policymakers of its expertise and availability and shall counsel those agencies and persons upon their request. The SSAC shall adhere in particular to the guidelines described in Article V, Section 2 of the Constitution.
- Section 11. *Ad hoc Committees*. The President may appoint such additional committees as may be needed to carry out the functions of the Academy. Ad hoc committees serve only during the tenure of the president who appointed them. Reports of ad hoc committees shall be presented to Council or to the annual meeting.

BYLAW 7. Publications

Section 1. *Editorial Committee*. Three regular members are appointed to the Editorial Committee for renewable three year terms. The Editorial Committee shall develop and recommend the Academy publication program and policies to the Council. It will assist the Editors of each official publication in reviewing manuscripts for those publications that include the *Proceedings*. Chairs of symposia will review manuscripts written for relevant symposia.

Section 2. *Managing Editor*. The Secretary-Treasurer shall serve as the

Section 3. *Editor*. Editors shall serve three year terms. The Editors shall edit all official publications of the Academy including the *Proceedings*.

#### BYLAW 8. Memorial Fund

The Council of the Academy shall establish a J. Donald Henderson Memorial Fund and administer this fund so that the proceeds will be used to promote science in North Dakota.

#### BYLAW 9. Fiscal Year

The fiscal year of the North Dakota Academy of Science, for the purpose of financial business, shall be 1 January to 31 December.

#### BYLAW 10. Achievement Award

The Academy establishes the North Dakota Academy of Science Achievement Award to be given periodically to an Academy member in recognition of excellence in one or more of the following:

- a. Nationally recognized scientific research.
- b. Science education.
- c. Service to the Academy in advancing its goals.

The Nominating Committee will administer the selection process, will develop a separate funding source for a monetary award, and will develop, for Council approval, the criteria for the award.

# BYLAW 11. Research Foundation

The **North Dakota Science Research Foundation** is established as an operating arm of the Academy. The purposes of the Foundation are:

(1) to receive funds from grants, gifts, bequests, and contributions from organizations and individuals, and (2) to use the income solely for the making of grants in support of scientific research in the State of North Dakota. Not less than 50% of the eligible monies received shall be placed in an endowment from which only the accrued interest shall be granted.

The foundation shall be responsible for soliciting the funds for the purposes described. The Foundation funds shall be in the custody of the Secretary-Treasurer of the Academy and shall be separately accounted for annually. The Foundation Board of Directors shall be comprised of five members of the Academy, representing different disciplines. Members shall be appointed by the President of staggered five year terms. The chairperson of the Board shall be appointed annually by the President. The Board shall be responsible for developing operating procedures, guidelines for proposals, evaluation criteria, granting policies, monitoring procedures, and reporting requirements, all of which shall be submitted to the Executive Committee for ratification before implementation.

The Foundation shall present a written and oral report to the membership of the Academy at each annual meeting, and the Secretary-Treasurer shall present an accompanying financial report.

# BYLAW 12. Affiliations

The Academy may affiliate itself with other organizations which have purposes consistent with the purposes of the Academy. Such affiliations must be approved by the Council and by a majority of those attending a regularly scheduled business meeting of the membership.

# BYLAW 13. Indemnification

Section 1. Every member of the Council or employee of the North Dakota Academy of Science shall be indemnified by the Academy against all expenses and liabilities, including counsel fees, reasonably incurred or imposed upon him/her in connection with any proceedings to which he or she may be made part, or in which he or she may become involved, by reason of being or having been a member of the Council, or employee at the time such expenses are incurred, except in such cases wherein the member of the Council or employee is adjudged guilty of willful misfeasance or malfeasance in the performance of his or her duties. Provided, however, that in the event of a settlement of the indemnification herein shall apply only when the Council approves such settlement and reimbursement as being for the best interests of the Academy. The foregoing right of indemnification shall be in addition to and not exclusive of all other rights to which such members of the Council or employee may be entitled.

# MINUTES (UNAPPROVED) OF THE NORTH DAKOTA ACADEMY OF SCIENCE **ANNUAL BUSINESS MEETING 2008**

University of North Dakota, Grand Forks, North Dakota, April 24, 2008, 12:00 PM

The first order of business was to approve the minutes of the previous business meeting from the April 2007 annual meeting in Minot, North Dakota.

A brief financial report was presented by Secretary-Treasurer Detke. At this time, the Academy is financially sound. Secretary-Treasurer Detke noted, however, that a large fraction of the membership that did not register for the 2008 meeting failed to pay dues for 2007 despite repeated reminders by email.

Paul Lepp, Minot State University volunteered for President-Elect in 2009. He was nominated and elected without opposition by voice vote.

Mike A. Davis, Nort nominated and elected		1 /	volunteered to serve as the third Council	or. He was
Meeting statistics:	140 0	Registered attendees Guests	55 professional, 85 student	

We had 11 professional talks and 47 Denison papers presented, of which 28 were graduate and 19 were undergraduate.

## A. Rodger Dennison Award winners:

Senior Division Yuhuoi Jin Graduate category:

Junior Division Amy Moritz

Undergraduate category: Brian Nelson

Paul Selid

# A. Rodger Dennison Award runner ups:

1<sup>st</sup> runner-up Senior Division: Susan Austin Graduate category:

> 1<sup>st</sup> runner-up Junior Division: Amali Samarasinghe 2<sup>nd</sup> runner-up Senior Division: Shibichakravarthy Kannan 2<sup>nd</sup> runner-up Junior Division: Ebot Tabe and Marron Bingle

Undergraduate category:  $1^{st}$  runner-up: Dennis Ingold  $1^{st}$  runner-up: Erikka Anderson  $2^{nd}$  runner-up: Andrew J. Hager 2<sup>nd</sup> runner-up: Jenna Wald

Van Doze (University of North Dakota) officially ended his duties as President by introducing Birgit M Prüß (North Dakota State University). President Prüß discussed preliminary plans for the Academy's 101st Annual Meeting, over which she will preside in Fargo on April 30, 2008.

The business meeting was adjourned at 1:00 PM.

# 2009 Dennison Award Winners Graduate Division



Winner: Yuhuoi Jin

PREPARATION OF LUMINESCENT SILICA NANOPARTICLES WITH CONTROLLABLE SIZES. Julia Xiaojun Zhao, Yuhui Jin

Department of Chemistry, University of North Dakota





Winner: Amy Moritz
THE N-TERMINAL TAIL OF THE DOPAMINE TRANSPORTER IS
PHOSPHORYLATED AT MULTIPLE SITES IN VITRO AND IN VIVO.
A. E. MORITZ, B. K. GORENTLA, R. A. VAUGHAN
Department of Biochemistry and Molecular Biology, University of North
Dakota,

1<sup>st</sup> runner-up: Susan Austin

APP MODULATES ENDOTHELIAL PHENOTYPE WITHIN THE VASCULATURE: IMPLICATIONS FOR ATHEROSCLEROSIS. Susan A.

Austin and Colin K. Combs

Department of Pharmacology, Physiology & Therapeutics, University of

North Dakota



Picture not available

1<sup>st</sup> runner-up: Amali Samarasinghe

EOSINOPHILIA AND IMMUNOGLOBULIN A IN EXPERIMENTAL ALLERGIC ASTHMA. Amali Samarasinghe\*, Scott Hoselton, and Jane Schuh.

Department of Veterinary and Microbiological Sciences, North Dakota State University



2<sup>nd</sup> runner-up: Ebot TAbe EVALUATION OF FECAL DNA PURIFICATION METHODS FOR THE DETECTION OF *E. COLI* O 157:H7 IN FECES OF NATURALLY INFECTED FEEDLOT CATTLE. Ebot S. Tabe, James Oloya, Dawn K.

Doetkott, Margaret L. Khaitsa. The Great Plains Institute of Food Safety, North Dakota State University

2<sup>nd</sup> runner-up: Marron Bingle
PALEOBIOGEOGRAPHIC ANALYSIS OF UPPERMOST
CRETACEOUS VIVIPARIDAE (CLASS GASTROPODA) FROM
INFRATRAPPEAN SEDIMENTS OF THE DECCAN PLATEAU,
INDIA. Marron Bingle and Joseph H. Hartman

Department of Geology and Geological Engineering, University of North Dakota,



2<sup>nd</sup> runner-up: Shibi Kannan ALVEOLAR MACROPHAGE PHAGOCYTOSIS AND RESPIRATORY BURST ACTIVITY IS REGULATED BY LYN -PI3K - AKT PATHWAY.. Shibichakravarthy Kannan\*, Aaron Audet, Huang Huang, and Min Wu.

Department of Biochemistry and Molecular Biology, University of North Dakota.

# 20078Dennison Award Winners Undergraduate Division



Winner: Brian Nelson RGS PROTEIN SUPPRESSION OF  $G\alpha_0$  PROTEIN-MEDIATED  $\alpha_{2A}$ -ADRENERGIC INHIBITION OF MOUSE HIPPOCAMPAL CA3 EPILEPTIFORM ACTIVITY. Brian Nelson, Ke Xu, Brianna Goldenstein, Elizabeth Luger, Jacquline Pribula, Jenna Wald, David Weinshenker, Raelene Charbeneau, Xinyan Huang, Richard Neubig, Van Doze. Department of Pharmacology, Physiology & Therapeutics, University of





North Dakota, Grand Forks, ND;

Winner: Paul Selid
DEVELOPMENT OF TARGET-INDUCED FLUORESCENT
NANOPARTICLES FOR THE DETERMINATION OF MERCURY. Paul D.
Selid, Song Liang, Hanying Xu, Julia Xiaojun Zhao
Department of Chemistry, University of North Dakota, Grand Forks, ND 58202

1<sup>st</sup> runner-up: Dennis Ingold SCALING UP THE ACCELERATED LEUCKART REACTION FOR THE SYNTHESIS OF NOVEL FORMAMIDE FUNGICIDES. Dennis Ingold and Mikhail M. Bobylev

Department of Biology, Minot State University





1<sup>st</sup> runner-up: Erikka Anderson GENETIC DIVERSITY AND SELECTION OF COMMON BEAN GENOTYPES FOR MAPPING GENES CONDITIONING MINERAL CONTENTS. Erika Anderson, Kayla Schmidt, Zahirul Talukder, Phillip Miklas, and Khwaja Hossain Mayville State University,.

2<sup>nd</sup> runner-up: Andrew J. Hager
DETERMINATION OF HEAVY METAL CONCENTRATIONS IN
SOIL SEDIMENT AND MUSSELS OF EASTERN NORTH DAKOTA
RIVER SYSTEMS . Andrew J. Hager\*, Louis M. Wieland, Andre W.
DeLorme.

Department of Biology, Valley City State University, Valley City,.





2<sup>nd</sup> runner-up: Jenna Wald
ALPHA-2 ADRENERGIC RECEPTOR INHIBITION OF HIPPOCAMPAL
EPILEPTIFORM ACTIVITY: COMPARISONS OF LIGAND EFFICACY
AND POTENCY, Jenna M. Wald, Brianna L. Goldenstein, Brian W. Nelson,
Ke Xu, Jacquline A. Pribula, Jasmine J. O'Brien, Kylie L. Davis, Kristan M.
Green, Sarah J. Boese, Jessica A. Lichter, James E. Porter, Van A. Doze
Department of Pharmacology, Physiology, and Therapeutics, University of
North Dakota

## AGENDA/NOTES

# Notes for the 101st Annual Business meeting

The first order of business was to approve the minutes of the 101 Meeting in Fargo, North Dakota, in 2009.

The minutes: were | were not approved as printed in the Proceedings | as amended.

A brief financial report was presented by Secretary-Treasurer Detke.

(Person to be name) has volunteered to be President in 2011. He | She was elected without opposition by voice vote.

Meeting statistics: \_\_\_\_ Registered attendees \_\_\_\_ () professional, () student \_\_\_\_ Guests

We had () professional talks and () Denison papers presented, of which () were graduate and () were undergraduate.

A. Rodger Dennison Award winners:

Graduate category:

Undergraduate category:

A. Rodger Dennison Award runner ups:

Graduate category:

Undergraduate category:

Birgit M Prüß (North Dakota State University) officially ended her duties as President by introducing Paul Lepp (Minot State University). President Lepp discussed preliminary plans for the Academy's 102<sup>nd</sup> Annual Meeting, over which he will preside in Minot on April (), 2009.

# **Executive Committee Membership**

President
Past-President
President-Elect
Secretary-Treasurer
Councilors (three-year terms)

#### **President**

Birgit M Pruess (2005-2008) Department of Veterinary and Microbiological Sciences North Dakota State University Fargo, ND (701)231-7848 Birgit.Pruess@ndsu.edu

# **President-Elect**

Paul Lepp Department of Biology Minot State University Minot, ND (701)858-3079 paul.lepp@minotstateu.edu

#### **Past-President**

Van Doze Department of Pharmacology Physiology & Therapeutics University of North Dakota Grand Forks, ND 58203 (701)777-6222 vdoze@medicine.nodak.edu

# Secretary-Treasurer

Siegfried Detke (2006-2009) Department of Biochemistry & Molecular Biology University of North Dakota Grand Forks, ND 58203 (701)777-3202 sdetke@medicine.nodak.edu

## **Councilors**

Douglas Munski (2004-2009) Department of Geography University of North Dakota Grand Forks, ND 58203 (701)777-4246 douglas\_munski@ und.nodak.edu

Jon Jackson (2007-2009) Department of Anatomy University of North Dakota Grand Forks, ND (701) 777-4911

Mike A. Davis (2008-2010) Environmental Scientist North Dakota Department of Transportation Bismarch, ND 58505 (701) 328-3704 madavis@nd.gov

## COMMITTEES OF THE NORTH DAKOTA ACADEMY OF SCIENCE

Executive Committee
Editorial Committee\*
Education Committee\*
Denison Awards Committee\*
Necrology Committee\*
Nominating Committee
Resolution Committee\*
Membership Committee\*
North Dakota Research Foundation Board of Directors\*

<sup>\*</sup> indicates available openings

# PAST PRESIDENTS AND THE LOCATIONS OF THE ANNUAL MEETING OF THE NORTH DAKOTA ACADEMY OF SCIENCE

1000	MAD	C IF I	1061	V F	C 1F 1
1909	M A Brannon	Grand Forks	1961	Vera Facey	Grand Forks
1910	M A Brannon	Fargo	1962	J F Cassel	Fargo
1911	C B Waldron	Grand Forks	1963	C A Wardner	Grand Forks
1912	L B McMullen	Fargo	1964	Fred H Sands	Fargo
1913	Louis VanEs	Grand Forks	1965	P B Kannowski	Grand Forks
1914	A G Leonard	Fargo	1966	Paul C Sandal	Fargo
1915	W B Bell	Grand Forks	1967	F D Holland, Jr	Grand Forks
1916	Lura Perrine	Fargo	1968	W E Dinusson	Fargo
1917	A H Taylor	Grand Forks	1969	Paul D Leiby	Minot
1918	R C Doneghue	Fargo	1970	Roland G Severson	Grand Forks
1919	H E French	Grand Forks	1971	Robert L Burgess	Fargo
1920	J W Ince	Fargo	1972	John C Thompson	Dickinson
1921	L R Waldron	Grand Forks	1973	John R Reid	Grand Forks
1922	Daniel Freeman	Fargo	1974	Richard L Kiesling	Fargo
1923	Norma Preifer	Grand Forks	1975	Arthur W DaFoe	Valley City
1924	O A Stevens	Fargo	1976	Donald R Scoby	Fargo
1925	David R Jenkins	Grand Forks	1977	Om P Madhok	Minot
1926	E S Reynolds	Fargo	1978	James A Stewart	Grand Forks
1927	Karl H Fussler	Grand Forks	1979	Jerome M Knoblich	Aberdeen, SD
1928	H L Walster	Fargo	1980	Duane O Erickson	Fargo
1929	G A Talbert	Grand Forks	1981	Robert G Todd	Dickinson
1930	R M Dolve	Fargo	1982	Eric N Clausen	Bismark
1931	H E Simpson	Grand Forks	1983	Virgil I Stenberg	Grand Forks
1932	A D Wheedon	Fargo	1984	Gary Clambey	Fargo
1933	G C Wheeler	Grand Forks	1985	Michael Thompson	Minot
1934	C I Nelson	Fargo	1986	Elliot Shubert	Grand Forks
1935	E A Baird	Grand Forks	1987	William Barker	Fargo
1936	LR Waldron	Fargo	1988	Bonnie Heidel	Bismark
1937	J L Hundley	Grand Forks	1989	Forrest Nielsen	Grand Forks
1938	P J Olson	Fargo	1990	David Davis	Fargo
1939	ED Coon	Grand Forks	1991	Clark Markell	Minot
1940	J R Dice	Fargo	1992	John Brauner	Grand Forks
1941	F C Foley	Grand Forks	1993	John Brauner	Jamestown
1942	F W Christensen	Fargo	1994	Glen Statler	Fargo
1943	Neal Weber	Grand Forks	1995	Carolyn Godfread	Bismark
1944	E A Helgeson	Fargo	1996	Eileen Starr	Valley City
1945	W H Moran	Grand Forks	1997	Curtiss Hunt	Grand Forks
1946	J A Longwell	Fargo	1998	Allen Kihm	Minot
1947	A M Cooley	Grand Forks	1999	Joseph Hartman	Grand Forks
1948	R H Harris	Fargo	2000	Mark Sheridan	Moorhead, MN
1949	R B Winner	Grand Forks	2001	Ron Jyring	Bismark
1950	R E Dunbar	Fargo	2002	Jody Rada	Grand Forks
1951	A K Saiki	Grand Forks	2003	Richard Barkosky	Minot
1952	Glenn Smith	Fargo	2004	Anna Grazul-Bilska	Fargo
1953	Wilson Laird	Grand Forks	2005	Holly Brown-Borg	Grand Forks
1954	C O Clagett	Fargo	2006	Andre Delorme	Valley City
1955	G A Abbott	Grand Forks	2007	Chris Keller	Minot
1956	H B Hart	Jamestown	2008	Van Doze	Grand Forks
1957	W E Comatzer	Grand Forks	2009	Birgit M Prüβ,	Fargo
1958	W C Whitman	Fargo	2007	211510 111 1 1up,	- 41-50
1959	Arthur W Koth	Minot			
1960	H J Klosterman	Fargo			
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Sempra Energy (Dividend)	\$295.50	
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Total	\$373.86	
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Sempra Energy (purchased as		
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Number of shares 250 (1983)	979.586	
Price 18.50	\$56.14	
Value \$4,625.00	\$55,082.13	
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Number of shares 120 (1990)	254.461	
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# Agenda for Business meeting Thursday April 30, 2007

- 1) Approval of the minutes for the 2008 100<sup>th</sup> Annual Meeting in Grand Forks
- 2) Old business
- 3) Election of President-elect
- 4) Election of Secretary/Treasurer
- 5) Other new business
- 6) Adjourn