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ABSTRACTS

Abstracts are listed alphabetically by the last name of the first author listed.

HYDROGEN SULFIDE AND ITS EFFECT ON CELLULAR METABOLISM

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We have studied the effects of varied concentrations of hydrogen sulfide (H₂S) on a line of human prostate cancer cells (22RV1). We are investigating the effect of H₂S over time in order to see if the results are both reproducible and reversible. Literature has reported that the physiological effects of H₂S are 100% reversible. Hydrogen sulfide is an endogenously produced gasotransmitter, but we are interested in exposing cells to higher concentrations than are typically produced in the body. Metabolic rate is to be measured by a fluorescent ATP assay and, based on literature, should greatly decrease as H₂S concentrations increase.

ANALYSIS OF PROTEIN EXPRESSION DURING REGENERATION IN *Lumbriculus variegatus*

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Regeneration of body parts in the freshwater oligochaete *Lumbriculus variegatus* (California blackworm) occurs via morphallaxis and epimorphosis. To learn about cellular and molecular aspects of epimorphic regeneration in *L. variegatus*, we carried out experiments using a proteomics approach. Cell homogenates were made of regenerating tissues harvested at 3, 7, 10, and 14 days into regeneration as well as from non-regenerating tissue. The extracted proteins were separated using two-dimensional gel electrophoresis. Gels were scanned and analyzed using Dymension 2 software to determine differentially expressed proteins. Protein profiles from regenerating heads were compared with those from regenerating tails. Most proteins were present in both head and tail blastemas. Mass spectrometric analysis of two such prominent proteins provided evidence that these proteins are components of the extracellular hemoglobin from *L. variegatus*. With increasing time into regeneration, the majority of the proteins were still common to regenerating heads and tails, but some proteins specific to either heads or tails emerged. We speculated that these proteins, differentially expressed in head or tail segments at the completion of regeneration, are structural proteins from these anatomically distinct regions of *L. variegatus*.

A comparison of temporal changes of protein expression in newly formed head and tail tissue was also performed. Compared with protein profiles from non-

regenerating tissues, several proteins were up-regulated at days 3, 7, and 10 with maximum expression seen at day 7. Three of these proteins with molecular weights of 51,000, 70,000, and 85,000 daltons were observed in both heads and tails. We speculated that these proteins may be candidates for involvement in epimorphic mechanisms in *L. variegatus*. A further analysis of these proteins by mass spectrometry is anticipated.

CHARACTERIZATION OF ZEB-1 REGULATORY ELEMENTS IN THE PCa CELL LINE 22Rv1

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The final stage of cancer progression, metastasis, is the most deadly and is primarily responsible for the lethality of human carcinomas. The determination of molecular biomarkers to predict advancement to this stage is crucial in the fight against cancer mortality. A potential biomarker for the onset of metastasis in prostate carcinoma (PCa) is the transcription factor Zinc finger E-box Binding Protein-1 (ZEB-1). ZEB-1 may prove a more valuable tumor marker than the conventional, yet nonspecific, prostate-specific antigen (PSA) protein. ZEB-1 is involved in human reproductive cancers, is known to be regulated by female sex steroid hormones, and is possibly bound by the tumor suppressor protein p53.¹ ZEB-1 has also been found to be regulated by androgens and is associated with the progression of PCa.¹ Additional investigation is required to more completely elucidate ZEB-1 regulation, determine its relationship to PCa etiology, and validate its usefulness as a metastatic biomarker.

In this study, we utilize the PCa cell line 22Rv1 to investigate relative ZEB-1 expression levels in response to a variety of putative regulatory factors. We employ the highly sensitive method of quantitative real-time polymerase chain reaction (qRT-PCR) to measure relative gene expression. The established positive control gene PSA and the newly identified negative control gene ribosomal protein L4 (RpL4) are used as internal references in the 22Rv1 system. Our results demonstrate that ZEB-1 expression is induced by several agents, among them dihydrotestosterone (DHT) and its metabolic breakdown products.

¹ Anose, B. M.; Landry, M. M.; Sanders, M. M. In *Investigating the Role and Regulation of ZEB-1 by Quantitative Real-Time PCR*; International Proceedings of the 12th International Congress of Endocrinology; Medimond: Bologna, Italy, 2004; pp. 1473-1478.

IDENTIFICATION OF AN ALLOSTERIC BINDING SITE IN GAMMA-GLUTAMYL-CYSTEINE LIGASE

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Glutathione has been implicated in chemotherapeutic resistance due to its function in conjugation and detoxication of electrophiles and its high abundance in cancer cells. By inhibiting glutathione synthesis selectively in cancer cells, these cells may become depleted of glutathione and more susceptible to chemotherapeutic agents. The γ -GCL catalyzes the first and gamma enzyme gamma-glutamylcysteine ligase (rate-limiting step in the synthesis of glutathione) and is therefore an appropriate target for inhibitor design.

In the present study, our objective was to identify small molecules that alter enzyme activity as a function of gamma-aminobutyric acid concentration, used here as an analog of the natural substrate, cysteine. The small molecules used were non-substrate amino acids. Our results show that most of the non-substrate amino acids examined inhibit the enzyme at high, near-saturating concentrations of gamma-aminobutyric acid but activate the enzyme at low concentrations of gamma-aminobutyric acid. Fluorescence spectroscopy was used to probe the effect of these non-substrate amino acids on the binding of substrates. Resultant plots of non-substrate concentration versus fraction protein bound are sigmoidal in shape rather than the typical hyperbolic shape characteristic of most monomeric enzymes. Additionally, dissociation constants increase for each non-substrate amino acid in the presence of increasing concentrations of gamma-aminobutyric acid.

Our results suggest that the enzyme contains at least two distinct binding sites. We hypothesize that these sites may bind either substrate or a non-substrate and that the binding of a molecule in one site impacts binding in the other site (i.e. the two sites have an allosteric relationship). Further development of binding sites within gamma-GCL have been completed in the presence of cobalt, indicating specific binding of two equivalents of cobalt, followed by non-specific binding at higher equivalents. The data will aid future NMR studies in the presence of cobalt. This work was supported through the Greater Gustavus Fund, Langsjoen Fund, and Sigma Xi Fund at Gustavus Adolphus College.

IMMUNOLocalIZATION OF ACTIN IN TRANSGENIC AND WILDTYPE MURINE MYOCARDIUM

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In myocardium, actin and myosin filaments are organized into repeating units of sarcomeres, the basic unit of muscle contraction. Actin Capping Protein (CP) binds to the barbed ends of the actin filament at the Z-line, directing and maintaining the proper organization of the thin filament in the sarcomere. CP is a heterodimer composed of an alpha (α) and a beta (β) subunit. Muscle cells contain two β subunit isoforms, $\beta 1$ and $\beta 2$. The $\beta 1$ isoform is present at the Z line; the $\beta 2$ isoform is found elsewhere including cell-cell junctions. In previous studies, transgenic mice were generated that replaced the $\beta 1$ isoform with the $\beta 2$ isoform. We hypothesized that a decrease in $\beta 1$ expression will lead to a disorganized myofibrillar structure and that the disorganization will become increasingly severe as a function of murine age. We examined the myocardium of transgenic mice ranging in age from three months to twelve months. Murine hearts were extracted and frozen sections prepared using a cryomicrotome. The tissue sections were fixed, quenched with ethanolamine, permeabilized with methanol, and washed in phosphate buffered saline. The sections were probed with mouse anti-actin-Cy3 rhodamine conjugated antibody and images captured using an Olympus fluorescent microscope and Simple PCI 6 acquisition software with deconvolution. Three month myocardium exhibited minor disorganization of the Z-lines compared to six, nine, and twelve month myocardium. The Z-lines in the twelve month myocardium were grossly disorganized with irregular breaks. In addition, the spacing between Z-lines was variable indicative of altered actin filament length.

NOBLE METAL NANOPARTICLE LOCALIZATION IN MAST CELLS

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Nanoparticle use in consumer products and medical treatments is rapidly increasing based on the unusual chemical and physical properties of nanoscale materials. If these nanoparticles are to be implemented in biological systems, it must be seen first how the cells of the human body react to the presence of nanoparticles. An important consideration is how the nanoparticles are taken up by endocytosis and where they reside once in the cell.

In this research, primary culture mast cells were exposed to serum-coated Au nanoparticles at concentrations of 1.0 nM, 0.1 nM, and 0.01 nM and for

varying exposure times of 24, 48, and 72 hours. Cells were then imaged using transmission electron microscopy (TEM). It is clear from these images that the nanoparticles are congregating mainly in the acidic secretory granules, the compartment of the mast cell that contains the chemical messengers serotonin and histamine. Using TEM images, the number of nanoparticles per granule was quantitated as was the size of the nanoparticles and the size of the granules before and after uptake. Cells exposed longer took up more nanoparticles than those exposed for short periods, but high-concentration nanoparticle solutions tended to aggregate, disrupting the endocytosis process and causing fewer nanoparticles to enter the cell. In parallel studies, electrochemical detection of the secreted chemical messengers demonstrated that the amount endocytosed was influenced by nanoparticle exposure time and concentration.

POPULATION-LEVEL INTERACTIONS BETWEEN TALL GOLDENROD (*Solidago altissima*) AND A STEM-GALLING FLY (*Eurosta solidaginis*)
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The stem-galling fly *Eurosta solidaginis* lays eggs on its host plant tall goldenrod *Solidago altissima* in May. The gall fly larva forms a spherical growth in the plant stem about the size of a quarter, which is referred to as a ball gall. The gall provides the larva with nutrients until it pupates and emerges as an adult in the spring. Goldenrod can reproduce asexually through rhizomes, resulting in clones of genetically identical plants or ramets.

In October we marked 150 goldenrod clones in a large open field in Brooklyn Park, MN. For each clone we measured the diameter, the number of flowering and non-flowering plants, and the height of the five tallest ramets. The number of ball galls was recorded and galls/m² was derived. The presence of rosette galls was also noted. Rosette galls are produced by the rosette midge fly *Rhopalyomia solidaginis*. Clones with few galls are relatively resistant to gall fly oviposition and/or survival of larvae. Ball galls were present in 72% of clones. Rosette galls were found in 28% of clones and were never abundant within clones. When comparing resistant and susceptible clones, we found that clone area was positively correlated with number of ball galls and plant density was negatively correlated with number of galls. Clones with numerous ball galls had taller and faster growing plants. *Eurosta solidaginis* apparently did not choose some clones for oviposition even though the physical characteristics of the clone were similar to those of nearby susceptible clones.

EFFECTS OF RESTORATION ON BIOGEOCHEMICAL PROCESSES IN RESTORED TALL-GRASS PRAIRIES

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In recent decades, prairie restoration projects have become commonplace in many areas that were once primarily agricultural fields. We are interested in how biogeochemical processes change as a restored field ages and becomes an established prairie ecosystem. Additionally, we are interested in how fire may affect biogeochemical processes of restored fields.

To answer these questions, we studied six fields, including a chronosequence of five restored plots on the campus of St. Olaf College in Northfield, MN, and one agricultural field. Plots ranged from 3 to 18 years since restoration, with a cultivated soybean field serving as age 0. Time since burn for plots ranged from 0.5 to 3.5 years. In each restored plot we measured soil CO₂ flux, above- and below-ground biomass, nitrification and N mineralization, microbial C:N, percent moisture, percent C and percent N in the soil, and total organic matter. Total organic matter, percent moisture, and percent C all decreased with time since restoration, while soil CO₂ flux and total microbial C:N increased with time since restoration. Our data strongly suggest nitrogen limitation of biological processes in all restored prairies. C:N ratios of microbes increased as a function of time since restoration, and plant C:N increased with time since previous burn, likely due to a decline in relative N availability. Percent N, nitrification, and mineralization did not show significant temporal patterns, but did have significant spatial variation. Our data suggest that time since burn has a strong influence on above-ground variables while age since restoration influences below-ground variables.

THE IMPACT OF COLLAGEN WOUND DRESSINGS IN THE PREVENTION OF ALVEOLAR OSTEITIS AFTER COMPLETE BONY IMPACTED MANDIBULAR THIRD MOLAR SURGERY

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In this study, we compared the incidence of alveolar osteitis (AO) in patients treated with CollaCote®, an absorbable collagen wound dressing. The purpose of this study is to determine whether CollaCote® is a substantial treatment in the prevention of alveolar osteitis.

Forty-two patients, classified with complete bony impacted mandibular third molars, were included in this study.

During surgery, CollaCote® was placed in one of the two vacant sockets in the mandible; the other socket that did not receive CollaCote® acted as a control group for our study. The same surgeon treated all patients. Results show that 31.0% (13 of 42) of the patients developed AO in one or both sockets following treatment. Of the 31.0% with AO, 92.3% (12 of 13 patients) developed AO in the socket treated with CollaCote®, while only 76.9% (10 of 13) developed AO in the socket left untreated (control). These results suggest that CollaCote® does not significantly impact the prevention of AO after third molar surgery.

THE PHYSIOLOGICAL RESPONSES OF *Lepomis gibbosus* AND *Mus musculus* TO *Noturus gyrinus* TOXIN

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Noturus gyrinus, a fish commonly known as the tadpole madtom, is a member of the catfish family Ictaluridae. The tadpole madtom produces a toxin that is associated with its pectoral spines, and this toxin is speculated to serve as a protective mechanism against predators including fishes and possibly some mammalian species.

A recent study at St. Mary's University determined that *Lepomis gibbosus* (pumpkinseeds) injected with tadpole madtom toxin experienced paralysis as well as a color change at the injection site that spread to varying degrees across the body surface. Interestingly, pumpkinseeds injected a second time with toxin were paralyzed a significantly shorter period of time following the second injection compared with the first. These results suggest that the pumpkinseeds may have developed an immune response to the toxin, thus reducing the physiological effects the toxin had on the fish.

More recent research at St. Mary's University indicates that tadpole madtom toxin also has a physiological effect on mice (*Mus musculus*). Mice injected with a saline solution containing tadpole madtom toxin displayed significantly impaired mobility compared with mice injected with saline solution alone. Studies are presently underway to determine whether the physiological effect of tadpole madtom toxin on the mice is less dramatic following the second toxin injection compared with the first injection. Additionally, sera from the mice are being analyzed by Enzyme Linked Immunosorbent Assay (ELISA) to determine whether the mice produce antibodies specific for tadpole madtom toxin. These results will help to elucidate whether predators of tadpole madtoms become immune to the

effects of tadpole madtom toxin following repeated exposures.

THE EFFECTS OF OVEREXPRESSION OF NKX2.1 ON LUNG SPECIFIC GENES SP-C AND SP-B IN

Xenopus laevis

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Lung development is controlled by many different factors. Among these different factors, genes play an integral role in initiating and coordinating lung development. Specifically, Nkx2.1 is a transcription factor that has been shown to be integral in the initiation of lung development. Much of the research done thus far with Nkx2.1 has been done on mice or rats and the effects of the gene are still largely unknown. However, when Nkx2.1 was absent in mice and rats, the organisms died at birth. Our study attempted to gain further knowledge into the role of Nkx2.1 through the process of overexpression of the gene in a new/different model organism, *Xenopus laevis*. Real-Time PCR results showed that when Nkx2.1 was overexpressed in *Xenopus* embryos the expression of the lung specific gene SP-C also increased. This suggests that Nkx2.1 has an effect on lung development and specifically other genes that are involved with lung development. It also suggests that when Nkx2.1 is increased in an organism other genes in that pathway are also increased.

RESPONSE OF FISH PREDATORS TO TADPOLE MADTOMS (*Noturus gyrinus*)

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The tadpole madtom *Noturus gyrinus* is highly prized as a bait fish in the upper Mississippi River Valley, where it is marketed as the "willow cat," yet it has venom glands associated with spines in its pectoral and dorsal fins.

The response of a predatory fish to this defense was tested by exposing tadpole madtoms to largemouth bass. As a control, results were compared with the response by bass to minnows, a common prey item for these predators. Each bass was used in six trials with minnows and six trials with tadpole madtoms. Trials using madtoms were alternated with trials using minnows. Largemouth bass were just as likely to consume tadpole madtoms as they were minnows (the prey was consumed in every trial). Bass sometimes took longer to handle and consume tadpole madtoms than minnows, but the likelihood of attack did not change as the number of trials increased.

THE EFFECTS OF LAURICIDIN ON THE ENTERIC BACTERIA OF *Mus musculus*

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Lauricidin, a dietary supplement, has been suggested to exhibit antimicrobial and antiviral capabilities in humans. It is unclear, however, what effect this dietary supplement has on the enteric bacteria in the gastrointestinal tract of individuals who are taking Lauricidin.

The purpose of this study was to examine the effects of Lauricidin on enteric bacteria, using mice (*Mus musculus*) as the animal model. One group of mice was treated with Lauricidin in a mixture of saline and peanut butter, and a second group was treated with a mixture of saline and peanut butter alone. Following six weeks of daily treatment, fecal matter was collected from the mice and was used as the source of enteric bacteria. The fecal material was suspended in saline, diluted in saline, and plated on tryptic soy agar and MacConkey's agar to quantify the numbers of total bacteria and gram-negative bacteria, respectively. After overnight incubation at 37°C, the colony-forming units (CFUs) on each plate were counted and recorded. These data were used to calculate the number of bacteria (total number and gram-negative bacteria) per gram of fecal matter. An ANOVA test was conducted to determine whether there was a statistically significant difference in the total number of enteric bacteria or gram-negative bacteria in the fecal matter of Lauricidin-treated versus control mice.

Results indicated there were no statistically significant differences in the total number of enteric bacteria or gram-negative bacteria present in Lauricidin-treated mice versus control mice. Thus, although Lauricidin may exhibit antimicrobial capabilities, it does not appear to influence the number or types of bacteria present in the gastrointestinal tract.

EXTRACTION AND IDENTIFICATION OF COMPOUNDS IN BITTER LEAF (*vernonia amygdalina*)

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The focus of this proposal was to isolate and determine the chemical composition of *Vernonia amygdalina* (VA) in both blanched and unblanched leaves. Chemical extractions were accomplished with four different solvents: methanol, hexanes, ethyl acetate and water. Two sets of extractions were performed. In the first set of extractions, the leaves were ground and allowed to stir in each of these solvents. Filtration was then used to remove extract from the solid remnant. In the second set,

a sohxlet extractor was used with the same solvents to further extract compounds from the solid remnant of the first extraction set. Thin layer chromatography (TLC) was used to separate the chemical constituents of the extraction. UV was used to identify organic compounds containing conjugated double bonds. Analysis of UV active compounds after TLC indicated that VA contains at least four methanol soluble compounds, one hexane soluble compound, six ethyl acetate soluble compounds and three water soluble compounds. No compound was detected in the aqueous extraction of blanched VA. Further analysis of extractions using LCMS is currently being conducted.

DIGGING UP GERMS: A PRELIMINARY INVESTIGATION OF THE BACTERIAL MICROFLORA IN SANDBOXES AT CHILDREN'S DAYCARE CENTERS

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In the realm of environmental health and medical microbiology, identifying sources and risk-factors contributing to illness in children is of great importance. Children are more susceptible to and severely affected by infectious disease.

The purpose of this preliminary study was to investigate two recent scientific claims which identified "playing in a sandbox" as a new risk-factor for contracting bacterial diseases. No similar previous research was found on this topic, suggesting the further importance and validity of the study. The goals of the research were to determine whether a sand environment supported microbial communities different from control groups, categorize microbial diversity between experimental locations, and identify possible pathogenic bacteria that might warrant further research in this area. Sand samples collected from multiple daycare centers were analyzed using culturable methods—using both differential and selective media to exploit biochemical differences between bacteria.

It was found that sandboxes from daycare centers not only support more diverse bacterial communities than do controls, but also sustain higher populations of those species. In addition to this, bacterial species suspect as pathogenic *Staphylococcus* sp. were identified in all sandboxes, and enteric coliform bacteria were present in all sandboxes and controls—suggesting possible implications for contamination points. Little is known about the public health consequences of contracting infectious diseases from sand. However, these data present a warranted concern and need for future research. The seasonal characteristics of microbial diversity, contamination processes, and length of bacterial survival in sand are possible directions for future research.

CONIFEROUS TREE SPECIES DURING SPRING THAW EVENTS IN SOUTHERN MINNESOTA

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The persistence of evergreen foliage throughout the winter gives conifers the ability to photosynthesize during warm winter periods and early spring when water is available. Typically photosynthetic rates are low in coniferous species compared to their deciduous counterparts. The ability to photosynthesize in less-than-optimal conditions may contribute to their annual net primary productivity.

Coniferous foliage is exposed to high visible and ultraviolet (UV) radiation, desiccating winds, and cold temperatures during the winter months in southern Minnesota. We examined photosynthetic performance and UV-screening ability of three coniferous species (*Pinus sylvestris*, *Picea pungens*, and *Pinus strobus*) on the campus of Minnesota State University–Mankato from January to April 2008 with modulated fluorometers. Plants showed very little photosynthetic ability during extreme cold events (-4 to -18°C) with light- and dark-adapted yields averaging <0.10 over all sampling dates. Concentrations of chlorophyll and carotenoids (carotenes and xanthophylls) were much higher in *P. sylvestris* and *P. pungens* than in *P. strobus*. In addition, concentrations of UV-absorbing phenylpropanoids were 63% greater in *P. sylvestris* and *P. pungens* than in *P. strobus*. The increased concentrations of these compounds may be related to the decreased epidermal transmittance of UV in the former two species. However epidermal transmittance of UV was appreciable. Epidermal transmittance of UV averaged 35, 52 and 62% for *P. sylvestris*, *P. pungens* and *P. strobus*, respectively.

We will continue to assess these parameters during the early spring and expect an increase in photosynthetic ability and a simultaneous reduction in UV transmittance as the season progresses and temperatures increase.

LEWIS ACID-MEDIATED COPOLYMERIZATION OF ACRYLAMIDES AND 1-HEXENE

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Controlled radical copolymerization of acrylamides and 1-hexene using the Lewis acids, Al_2O_3 and $\text{Sc}(\text{OTf})_3$, is expected to give copolymers with macroscopic properties that are unique when compared with the homopolymers.

This research studies the effect of the Lewis acid, Lewis acid concentration, and temperature on copolymer conversion and composition of each comonomer.

Characterization of the copolymers is achieved using ^1H NMR to determine copolymer composition, and GPC is used to analyze molecular weights and molecular weight distribution. It was found that temperature affects acrylamide copolymer conversion whereas the use of Al_2O_3 as a Lewis acid does not. However, $\text{Sc}(\text{OTf})_3$ was shown to increase copolymer conversion and significantly increase molecular weight of copolymers. The use of $\text{Sc}(\text{OTf})_3$ also showed an increase of 1-hexene incorporation while simultaneously affecting the tacticity of the copolymer.

WHAT DOES JANUS TYROSINE KINASE (JAK) 3 INHIBITOR DO TO T CELLS *IN VITRO*?

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T cells are the crucial players in autoaggression against insulin-producing beta cells that results with a development of autoimmune type 1 diabetes (T1D). There are subtypes of T cells that exhibit protective and pathogenic roles in the beta cell destruction. They can be distinguished by their surface molecular markers as well as by the specific cytokine profiles. T cells express cytoplasmic signal transduction molecule JAK3. It was found that the *in vivo* inhibition of JAK3 by a specific inhibitor WHI-P131 protected NOD mice (a mouse model of T1D) from diabetes development.

The goal of this study is to examine the effects of WHI-P131 on the survival, mechanism of cell death, and cytokine secretion of an isolated subpopulation of CD4+ T cells, in order to get an insight into the possible mechanism of WHI-P131 action. The CD4+ T cells were isolated from the spleens of 5-7-week-old NOD and control C57BL/6 mice by positive magnetic separation. The cells were exposed to three concentrations (6, 3, and 1.5 $\mu\text{g}/\text{ml}$) of WHI-P131 and cultured for the 3-week-culture period. The apoptotic cell death was determined in cell lysates, while cytokine secretion (IL-10, IL-4, IL-2, IFN-gamma and TGF-beta) was analyzed in cell supernatants. It is found that WHI-P131 induces a dose-dependent apoptotic cell death of the CD4+ T cells, clearly confirming that WHI-P131 indeed affects CD4+ T cells. It is expected that cytokine profiles obtained from the WHI-P131-exposed CD4+ T cells would provide an insight about the subtypes (protective vs. pathogenic) of CD4+ T cells induced/affected by WHI-P131 action.

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SAHARAN AIR LAYER VERTICAL PROFILES AS OBSERVED DURING NAMMA

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The Saharan Air Layer (SAL) is a layer of air and dust that originates in the Saharan Desert and is advected across the Atlantic Ocean. It is generally characterized by warm, dry air and layers of increased dust concentration and may play a major role in suppressing tropical cyclone development. In order to better understand the SAL, the NASA African Monsoon Multidisciplinary Analysis (NAMMA) airborne field campaign, stationed in Cape Verde, collected extensive SAL data during August and September, 2006.

In this study, the data set is closely examined in order to characterize the vertical structure of the SAL in terms of thermodynamic structure and dust loading. A comparison of vertical profiles obtained from non-SAL sampling and SAL sampling is conducted to identify the characteristics unique to the SAL. It is found that there is increased temperature and wind speed within the dust layer profiles. The SAL is also investigated as it is transported from the Sahara into the eastern Atlantic Ocean to better understand how the dust layer height, thickness, and moisture levels change during the transport processes.

The relationship between SAL and moisture is closely examined through the correlation between increased dust loading and moisture levels. The areas of increased dust loading are typically found between altitudes of 1 km to 6 km and their thicknesses range from 0.1 km to 2 km. Initial findings from this study show some dust layers contain high levels of moisture. This is contrary to previous understanding of Saharan dust layers.

DETERMINATION OF THE ELECTROCHEMICAL MECHANISM OF 2,4-DINITROTOLUENE IN AN AQUEOUS ENVIRONMENT

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This study is being performed to support a project that develops gas sensors for the detection of explosives. For this purpose, the electrochemical properties of 2,4-dinitrotoluene in water were investigated. Cyclic voltammetry (CV) shows six distinct peaks of interest; two at approximately -650 mV and -750 mV arising from the irreversible reduction of the nitro groups of 2,4-dinitrotoluene. Two peaks near -150 mV are associated with the quasireversible oxidation of a yet unknown compound that is only observed upon reduction of 2,4-dinitrotoluene; the quasireversibility is evident from the associated two reduction peaks at -190 mV. The

goal of further research is to determine the pH dependence of the electrochemical properties of 2,4-dinitrotoluene, and the identification of the unknown intermediate species.

THE PREDICTIVE VALIDITY OF NURSING ADMISSION MEASURES: A META-ANALYSIS

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Every year baccalaureate nursing educators are faced with the daunting task of reviewing many applications and deciding which prospective nurses will be successful in their program. A critical outcome is passing the licensure test, the NCLEX-RN. This creates two key needs. The first is selecting future students who are more likely to pass. The second is identifying and developing current students who are at risk of failure. A host of predictors has been proposed. Although many have studied the efficacy of different admission and diagnostic measures, small sample sizes limit the interpretability of the research literature and have led to inconsistent findings. We use meta-analysis to statistically combine the studies, thus raising the overall sample size and providing an opportunity to examine likely sources of study variability.

Meta-analyses were conducted on the predictive validities of ACT, SAT, pre-nursing GPA, overall nursing GPA, and nursing school GPA with the NCLEX-RN licensure test. The correlations were positive and sizable for all of the selected measures. Nursing grades were highly predictive of NCLEX-RN success eclipsing the commonly reported predictive validates for some assessment tests (e.g., NLN, Mosby). The strongest admissions predictor of NCLEX-RN success was the ACT social science score. The overall nursing GPA and the second year of nursing school GPA were the strongest predictors of success. Most of the variability in the literature appears to be due to sampling error rather than substantive moderators.

In conclusion, nursing GPA and nursing admission measures are very predictive of NCLEX-RN success. Development of additional predictors may improve the accuracy of admissions decisions. Further research is needed to examine other variables as well as examine the inter-correlations between variables.

TESTING PREDICTING CAPABILITIES OF HYDROLOGIC MODELS IN SPUNK CREEK WATERSHED, MINNESOTA

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Available hydrologic and hydraulic models such as HEC-HMS, HEC-RAS, WMS, and StormNet are used commonly in water resources, environmental assessment, and forecasts for precipitation-runoff events. These models work most proficiently scaling watersheds of smaller size which are less complex and often more homogeneous. However, there are always several intangibles that greatly impact the simulation results that may go unnoticed to the modeler. Among these are the effects of highly variable soils properties (which influence the degree of antecedent soil moisture and runoff potential, for example) and topography (in particular, topographic features at the scale of or smaller than DEM data resolution).

The hydrologic response of the Spunk Creek basin, MN (137 km²), which includes a series of complex glacial geomorphic features and post-glacial till, was monitored in the field and simulated using available hydrologic models. A temporary network of rainfall gauges was installed throughout the basin and resulting hydrographs from rainfall events were measured in the basin outlet. Field data were then used to test the predictive capabilities of HEC-HMS, HEC-RAS, and StormNET.

A parameter sensitivity analysis and model calibration suggests that small-scale complexity and variability can be accounted for in model parameters such as initial abstractions, basin slope, and lag time. The study aids not only in gaining a better understanding of the local hydrology of the Spunk Creek watershed but also in complex hydrologic and geologic environments.

TESTING FOR AROMATICS IN SNOW SAMPLES

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In 2002, Hennepin and Ramsey counties ranked in the highest 10% of all counties in the United States for air releases of recognized carcinogens. Minnesota releases approximately 3,000 pounds of polyaromatic hydrocarbons (PAHs) annually. In winter, the large amount of precipitation traps the free-floating aromatic compounds in the atmosphere and stores them. They are absorbed during the spring by different life forms and recycled, becoming more concentrated as they move up the food chain.

In this project, we examine the abundance of aromatic hydrocarbons trapped in snow samples. Preliminary results indicate that aromatic compounds are more prevalent in samples from certain areas than others.

SOLITON PROPAGATION IN DIFFUSIVELY COUPLED CHAOTIC DOUBLE PENDULA

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Chaotic systems are deterministic and sensitive to initial conditions. Solitons are waves that propagate at a constant speed and maintain their shape. This study considers the propagation of solitons in a chaotic medium, namely diffusively coupled double pendula. This study's hypothesis is that low energy waves will be able to propagate like classical solitons or approximately like classical solitons, but high energy waves will either dissipate or propagate in a chaotic way. The Runge-Kutta Fourth Order method was used to numerically solve the differential equations governing diffusively coupled single and double pendula. Systems of 100 single or 100 double pendula were simulated at various initial angles of the first pendulum and coupling strengths. The results were examined by looking at the index of the most energetic pendulum as a function of time. This data was compared to a triangle wave model, and the coefficient of correlation, or R^2 , was calculated to determine the coherence of the soliton. The hypothesis was supported, but only within the domain of this simulation: the single pendula system was able to support solitons of any energy, but the double pendula system was unable to support high energy solitons and became unstable instead. Unfortunately, the total energy of the high energy solutions increased many fold, perhaps due to insufficiencies in the numerical integration technique, or the extrapolation of the single pendula coupling force to the double pendula system. No conclusion about an actual physical system of double pendula coupled by springs could be made.

EFFECT OF NORMAL POLYGLUTAMINE POLYMORPHISM ON HUNTINGTON DISEASE PROTEIN FUNCTION

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Huntington Disease (HD) is a progressive neurodegenerative disorder that causes deterioration of specific brain cells, leading to uncontrolled movement, emotional disturbance, and ultimately death. HD results from a mutation in the DNA encoding the protein huntingtin—a large protein of unknown function ubiquitously expressed throughout all tissues of the human body. Interestingly, huntingtin contains a polyglutamine (polyQ) tract near its NH₂-terminus that is normally polymorphic within the human population (Q₆-Q₃₅), but that increases in size well beyond its normal range to cause the disease

It is unclear what consequence normal polyQ polymorphism has on huntingtin function within cells. To

shed light on this question, we are deriving a series of HD constructs differing in polyQ tract length. To date, we have in hand, huntingtin constructs with 23, 45, 55, and 86 glutamines. These constructs can ultimately be used to perform protein localization experiments within tissue culture cells to determine whether or not normal polyQ tract length polymorphism effects huntingtin intracellular localization.

MATHEMATICAL SIMULATION OF HUMAN VISUAL ACUITY

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Optical characteristics of the human eye may be modeled by an orthogonal polynomial series known as the Zernike series. We present a formulation that allows a Zernike representation of the eye to be calculated from second-order aberrations (i.e. spherical defocus and astigmatism), which are found in the ubiquitous eyeglass prescription. Using this representation, we calculate a convolution kernel, known as the point spread function (PSF), which may be convolved with an image to render

it as viewed through the optical system of that eye. The result is a systematic method for rendering a virtual scene viewed by a patient with given optical aberrations.

Qualitative behavior of our model agrees with intuition and the physical characteristics of light. This model can be easily extended to account for higher-order ocular aberrations, which may be measured by Shack-Hartmann aberrometry. Thus, this system may find practical applications in clinical ophthalmology, particularly in a qualitative pre- and post-operative analysis of a patient's vision.

SYNTHESIS OF N-HYDROXYSUCCINAMIDYL ESTERS OF FATTY ACID OMEGA-DISULFIDES

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Molecular scaffolds composed of nucleic acid are of great interest for development of nanometer scale devices. The common strategy for attaching things such as nanoparticles (NP's) to these scaffold structures involves reactions that occur, by necessity, in an aqueous environment. Unfortunately, these conditions may also lead to instability that ultimately causes the scaffold to fall apart. This fundamental problem poses a significant drawback in the development of fabrication processes that would otherwise take advantage of DNA's ability to self-assemble.

An alternative approach that may preserve scaffold structure would be to decorate a nucleic acid molecular scaffold with non-polar gold NP's at a

water/organic solvent interface. This strategy would retain the structure of the nucleic acid scaffold during NP decoration. To accomplish this, non-polar tether molecules were synthesized so that, when attached to a DNA scaffold, they will facilitate binding of gold NP's suspended in an organic solvent upon self-assembly. A series of omegamercapto-fatty acids was used to synthesize non-polar disulfide tethers of varying lengths. Ultimately these fatty acyl-disulfides will be coupled to an oligonucleotide and incorporated into a molecular scaffold, known as a G-wire. The disulfide groups of these G-wires act as non-polar tethers that can project into the non-polar solvent, and they will bind to gold nanoparticles in the aqueous layer across the polar/non-polar interface. These tethers will be tested for their effectiveness in mediating interfacial gold NP decoration by UV/Vis spectrophotometry and gel electrophoresis. It is expected that an optimal non-polar tether length will be found for efficient G-wire self-assembly and interfacial NP decoration.

LINKING PHOSPHATASE ACTIVITY TO NUTRIENT PHYSIOLOGY IN POPULATIONS OF THE N-FIXING CYANOBACTERIUM

Trichodesmium FROM THE SARGASSO SEA

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As the dominant nitrogen-fixing organism in tropical and subtropical oceans, *Trichodesmium* represents a significant source of new nitrogen in oligotrophic systems. Therefore, factors that influence the distribution and abundance of *Trichodesmium* have important consequences for biogeochemical cycling of carbon and nitrogen. One such controlling factor, dissolved inorganic phosphorus (DIP), is present in very low concentrations in regions inhabited by *Trichodesmium*. Dissolved organic phosphorus (DOP) is present in higher concentrations in these regions, but requires enzymatic hydrolysis to be readily used by most phytoplankton. The presence of these enzymes may represent an important adaptation for low-DIP environments.

The kinetics of DOP utilization can be examined using model substrates. In culture, phosphatase activity is linked to *Trichodesmium* DIP availability; cultures grown with low DIP show elevated phosphatase activity. A series of cruises through the Sargasso Sea has been undertaken to measure the activity of phosphatase, and results have been largely consistent with measured DIP concentrations across the transect. These results help to elucidate the role of DOP in the phosphorus budget of *Trichodesmium*.

COMPARATIVE STORAGE CAPACITY IN FOUR FREEZING CONDITIONS OF HEP₂, M₂, AND VERO CELLS

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Determining the storage capacity of tissue culture cells is important, as they are very expensive. Thus, the study improved financial feasibility of further research.

Human epithelial placenta (HEP₂), mouse melanoma (M₂), and African Green monkey kidney (Vero) cells were grown to monolayer at 37°C and 5% CO₂ tension in Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal calf serum and 1% penicillin/streptomycin sulfate in Falcon flasks. The cell lines were trypsinized and prepared for storage at the following freezing temperatures: -4°C, -20°C, -80°C, and liquid nitrogen. Initial cell viability was reduced by about 7% one hour post cryopreservation. At weeks 2, 4, 6, and 28, cell lines stored at different freezing temperatures were evaluated for viability by a live-to-dead cell ratio of stained cells with a hemacytometer. All lines stored at -4°C lost the ability to reform a monolayer after two weeks.

Results suggest that liquid nitrogen was the most effective for storage, with a 46.5% average survival rate at seven months, compared with 30.0% in -80°C. The mean time for monolayer formation stored in liquid nitrogen increased from 28 hours at week 2 to 128 hours at week 28. The growth to monolayer of M₂ cells was the quickest proceeded by Vero, then by HEP₂ after thawing from all freezing temperatures. Further work is in progress to both determine longest possible storage duration of cells and test birch bark extracts against melanoma cells.

TRANSCRIPTIONAL REGULATION OF OUTER MEMBRANE PROTEASE (ompT) in *Escherichia coli*

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OmpT proteins are a family of microbial outer membrane proteases found in Gram-negative Enterobacteriaceae. These proteins are functionally diverse; their biological functions include cell adhesion, microbial pathogenesis, and housekeeping proteolysis. Most research on ompTins has focused on the substrate specificity of these proteases and their role in microbial virulence. Little is known about the cellular and environmental cues that control the expression and activity of these proteases. Knowledge about the regulation of *ompTins* is important to get better insights

into their physiological functions and their roles in virulence.

The purpose of this study was to learn about the cellular factors and environmental cues that regulate the expression of ompT genes. In order to accomplish our goal, we studied the transcriptional regulation of protease VII (ompT). This protein is the most thoroughly studied member of the ompT family and the major housekeeping protease found in *Escherichia coli* cells. Bioinformatic analyses were performed to extract the regulatory regions of the *ompT* gene and its homologs in other species. Motif analyses identified consensus sequences for a variety of transcriptional regulators, including the Leucine Responsive Protein (Lrp). To verify the bioinformatics predictions, transcriptional profiles of wildtype *E. coli* and mutants cells lacking the leucine responsive protein were analyzed. The transcriptional profile data was confirmed using a reporter construct containing the GFP gene under the control of the *ompT* promoter. These studies revealed that gene expression driven by the *ompT* promoter occurred only in wildtype *E. coli* cells and was negligible in isogenic *lrp* mutants. These results suggest that Lrp is a transcriptional activator of *ompT* gene expression in *E. coli*.

CAPABILITIES OF GAS PHASE MICRODIALYSIS PROBES WHEN INTERFACED WITH PTR-MS, GC-FID, AND A NITRIC OXIDE ANALYZER

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Gas phase microdialysis probes have been used to extract volatile compounds in solution. The probes used are small (200 micron diameter), have a fast response time (around 1 sec), and can analyze both polar and non-polar compounds. Microdialysis probes have been interfaced with a proton transfer reaction mass spectrometer (PTR-MS) and have measured toluene at 0.01 mM, which is 100 times lower than the detection limit using a flame ionization detector.

Using the PTR-MS, we have seen improvement in the detection limits of other volatile organic compounds. The PTR-MS has been used to monitor a reaction in which 10 mM propanol is oxidized by dichromate. Microdialysis probes have also been interfaced with a GC-FID to monitor a reaction in which 200 mM ethanol is oxidized by dichromate. Using the GC-FID, we also studied the effect that solution temperature has on the probe's sensitivity. From 0° to 60°C ethanol's signal increased by a factor of 43, propanol's signal by a factor of 52, and toluene's signal by a factor of 4.

The effects that NaCl, protein, and acid in solution have on probe sensitivity were also studied. We found that NaCl and acid slightly increase the sensitivity for ethanol and propanol. Protein had little effect on the

sensitivity for these analytes. The sensitivity of toluene decreased slightly with NaCl and acid, and decreased by a factor of 17 with protein. Microdialysis probes have also been interfaced with Nitric Oxide Analyzer. NO has been measured in solution at 0.005 mM concentrations.

THE EFFECT OF XYLITOL AND SORBITOL ON

Streptococcus mutans GROWTH

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Bacteria in the human oral cavity thrive on the sugar found in the foods that we eat. *Streptococcus mutans* bacteria metabolize sugar and produce an acid that attacks tooth enamel. In recent years, higher levels of sugar consumption have increased the demand for the use of natural sugar substitutes in food and chewing gum. Xylitol and sorbitol are sugar alcohols that have recently become popular sugar substitutes in a variety of sugar-free gum such as Trident and Orbit. They cannot be metabolized by the bacteria in the mouth like sugar can.

A Minimum Inhibitory Concentration (MIC) test was conducted to assess the effects of different xylitol and sorbitol concentrations on *Streptococcus mutans* growth. It was found that neither xylitol nor sorbitol act as antiseptics. It was also found through the MIC test that the concentration of either sugar alcohol does not appear to have a direct effect on the growth of *S. mutans*. Further research in this area could help improve sugar-free chewing gum in the future.

A RECORD OF LATE PLEISTOCENE GLACIAL-INTERGLACIAL CYCLES: A PLANKTIC FORAMINIFERAL STUDY OF CARIBBEAN PALEOCEANOGRAPHIC SURFACE WATER CHANGES

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We used relative abundances of *Globorotalia menardii* to develop a biostratigraphic framework using Ericson Zones and to infer glacial-interglacial cycles over the past ~280 ky. The sediments studied were from a piston core (CH0288-PC-75) retrieved from a water depth of 1203 m, SE of the Pedro bank in the Caribbean. The highly temperature-sensitive planktic foraminiferal *Globorotalia menardii* complex is present and abundant in warm surface waters of today and is absent in cold surface waters today.

Our relative data show that Ericson zones Z-V are present. These zones indicate the past glacial-

interglacial periods that occurred over the time the core sediment was deposited. $\delta^{18}\text{O}$ data from this same core show that Marine Isotope Stages (MIS) 1-7 are present.

Together with the planktic foraminiferal data, these data suggest a possible shift in ocean circulation and ocean chemistry patterns over several glacial-interglacial cycles.

DEVELOPMENT OF THE PERSONAL SCIENCE TIMELINE: A MODEL OF EXPERIENCES AND RELATED ATTITUDES AND A TOOL FOR REFLECTION

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Research in education argues that how and how frequently we perform a given activity depends greatly upon attitudes toward and type of prior modeling of the activity we have observed. These assertions hold particular significance for those who provide educational experiences for learners in the area of science. One population of concern is future teachers. Research in science education related to elementary teachers suggests a high incidence of science anxiety, poor attitudes toward science, and issues with low self-efficacy related to teaching science. Uncovering the contributing variables poses a challenge, but this study hypothesized and found there is a relationship between the number and nature of prior science experiences and the attitudes and self-efficacy related to teaching science. The study reports on the development of a simple tool for examining prior experiences related to science and connected attitudes called The Personal Science Timeline: Memories and Attitudes. For this study, preservice elementary teachers enrolled in an instructional science methods course at a mid-size Midwestern university completed a timeline on which they were asked to record science memories across their life and gauge the related attitudes. A significant positive relationship was found between reported positive attitudes and those learning experiences having concrete components, built on prior knowledge, and providing analogies grounded in the learner's culture. Study continues related to the timeline as a model of development of attitudes and as a tool facilitating construction of educators' philosophies of how science is best learned and, therefore, how science should be taught.

CLONING AND INITIAL FUNCTIONAL CHARACTERIZATION OF UNKNOWN GENE KIAA1946

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In the early 1990s scientists from all over the globe embarked on a journey to unlock the human

genome, to find out how many genes lie twisting and tangling within our chromosomes. Nearly ten years later, the daunting task was complete; the 3 billion base pairs within our double-stranded DNA had been sequenced. Now, an equally immense task presents itself for scientists—to discover the cellular function of all of these hitherto unknown genes. In this regard, we have chosen to characterize the function of an unknown gene—KIAA1946. This gene is of interest to us because it has a polyglutamine tract within its primary amino acid sequence, and is likely expressed in the nervous system (several other polyglutamine proteins underlie a number of severe neurodegenerative disorders such as Huntington Disease)

As a first step in characterizing the cellular function of KIAA1946, we have successfully cloned it into pEGFPN1—a special vector that helps “tag” our novel protein with a fluorescent tracer called GFP (Green Fluorescent Protein). Our next step will be to transfect our KIAA1946-GFP fusion protein vector into tissue culture cells, and assay (using fluorescence microscopy) the normal cellular localization of KIAA1946. These data represent a first step in deciphering the normal cellular function of Kiaa1946.

THE DYNAMIC STRUCTURE OF HURRICANE RITA AND HOW IT COMPARES TO TROPICAL CYCLONE THEORY

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The exact process by which a tropical cyclone develops and maintains itself is still a question that remains unresolved. However, there are two competing theories that take precedence in explaining the atmospheric processes that intensify these storms. They are known as Conditional Instability of the Second Kind (CISK) introduced by Jule G. Charney and Arnt Eliassen and Wind Induced Surface Heat Exchange (WISHE) introduced by Kerry Emanuel.

The concepts behind these formation theories, which hold distinct profiles in their structural and internal processes, will be the focus of this study. With a composited set of dropsonde data taken from Hurricane Rita, thermodynamic and dynamical indices will be calculated to explore if either of these theories correlate with the actual processes that occur in nature.

ROBOTIC DISPERSION

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Robots are quickly becoming mainstream devices that are required to do jobs that are dirty, boring,

or dangerous for humans. With the ever-increasing need for robots to do these jobs, the need for more intelligent robots is growing. A specific task that humans have great interest in using robots for is reconnaissance missions. The military makes use of robots to go into potentially dangerous environments to map the environment and find items of interest such as wounded soldiers.

I'm investigating using small, inexpensive robots that use wireless intensity signals instead of sophisticated sensors and odometry to disperse within an unknown environment. Using wireless intensity signals as the primary way of communication, the robots can be kept very cheap and, pending results, I hope to see that a large swarm of small, inexpensive robots can more efficiently disperse and set up a sensor network within an unknown environment as compared with using large, expensive robots with sophisticated sensors.

STEREOCOMPLEX INTERACTIONS IN ENANTIOMERIC CYCLIC AND LINEAR POLYLACTIDES

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Zwitterionic polymerization of lactide can be accomplished using N-heterocyclic carbene (NHC) catalysts to yield linear and cyclic polymers of relatively high molecular weights and narrow polydispersities. Stereocomplexation behavior has been observed in mixtures of linear poly(L-lactide) (PLLA) and poly(D-lactide) (PDLA).

In this study, we sought to understand the role of topology on the formation of stereocomplexes. Here we provide evidence using differential scanning calorimetry (DSC) data for the existence of stereocomplexes between the linear and cyclic forms of polylactide. We show that stereocomplexes form readily between cyclic and linear polylactides. These studies will enable the synthesis and study of non-covalent assemblies from mixtures of cyclic and linear polymers.

CHARACTERIZATION OF NEUREGULIN/ERBB RECEPTORS IN NEONATAL RAT (*Rattus norvegicus*) DIAPHRAGM MUSCLE

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Neuregulin (NRG) is a nerve-derived trophic factor that has several functions in muscle signal transduction. NRG regulates cellular pathways through the binding of four ErbB receptors: EGFR (ErbB-1), ErbB-2, ErbB-3, and ErbB-4. NRG increases protein synthesis in C₂C₁₂ mouse myotubes and rat diaphragm in a PI3K manner which implicates NRG as a potentially important regulator. This study characterizes the presence of each of four ErbB receptors in neonatal rat diaphragm muscle (DIAM) to direct further research in determining the signaling pathways of NRG in the developing neonate. Rat DIAM at ages P₀, P₇, and P₂₈ was analyzed for each ErbB receptor via Western analysis. We compared the relative amounts of each receptor and observed a trend with the greatest expression for all receptors at P₀ and decreased expression through P₂₈. This data suggests a major role for NRG in stages of early development and differentiation.

SYNTHESIS AND CHARACTERIZATION OF SQUARE PLANAR METAL COMPLEXES OF THE LIGAND 2,2-DICYANOETHYLENE-1,1-DITHIOLATE

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Ionic transition metal complexes have emerged as favorable candidates for applications in solid-state electroluminescent devices. Electronic device applications employing molecular electronic materials include LEDs (light-emitting diodes) and LECs (light-emitting electrochemical cells). Coordinatively unsaturated square planar d⁸ metal complexes of Pd(II) and Pt(II) have great potential for use in electronic materials applications as they frequently possess desirable photophysical properties.

In an effort to develop new ionic transition metal salts, we studied the spectroscopic, electrochemical, and structural features of two complexes (TBA)₂[Pt(i-mnt)₂] and (TBA)₂[Pd(i-mnt)₂] (TBA = tetra-n-butylammonium, i-mnt = 2,2-dicyanoethylene-1,1-dithiolate). Electronic spectra were consistent with intra-ligand and charge-transfer character as well as the presence of d-d transitions.

The electrochemical behavior of the complexes can be explained as primarily ligand-based. Quantum mechanical calculations agree qualitatively with the electronic spectra and electrochemical data. Single-crystal X-ray structures verified the expected deviations from square planar geometry. Electrospray mass spectrometry and attenuated total reflectance infrared spectroscopy were used to additionally characterize these complexes. The results of this study will be used as a basis for further

exploration of heteroleptic Pd(II) and Pt(II) complexes containing i-mnt and organometallic ligands such as the phenylpyridine anion (ppy).

GENERATION OF RABBIT AND CHICKEN POLYCLONAL ANTIBODIES

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Actin, a cytoskeletal component of all eukaryotic cells, plays an important role in diverse cell functions including maintaining cell shape and contributing to cell motility. Actin filament length and stability are regulated by a variety of accessory proteins including actin capping protein (CP). In vertebrates, three alpha isoforms (α 1, α 2, α 3) and three beta isoforms (β 1, β 2, β 3) have been identified.

We hypothesize that the alpha isoforms have distinct functions in tissues and cells, which suggests that the proteins have different localization patterns. To evaluate the expression of the alpha proteins, we are generating alpha isoform-specific antibodies which will be used in future localization studies. Because alpha isoform antibodies do not exist, I am preparing two new polyclonal anti-mouse CP sera, one generated in chicken and one generated in rabbit, which will allow for double localization studies. The immunogens were peptides for mouse CP α 1 and CP α 2, one specific for mouse α 1 and one specific for mouse α 2. Fusion protein constructs were prepared in pGEX-6, a glutathione S transferase vector. The protein were expressed in *E. coli* and purified by affinity chromatography. The protein concentration was determined by Bradford analysis and used as an immunogen in both chicken and rabbit. We have determined the titer of the production antibodies using Western Blot analysis. The α 1 antibodies, generated in chicken, have a reactive titer of 10⁻⁶. α 2 antibody production in rabbit is underway.

FIXATION COMPARISON BETWEEN A CORTICAL-CANCELLOUS SUSPENSION TITANIUM SUTURE BUTTON BACKUP, RETROGRADE BIOABSORBALE SCREW, AND COMBINED USE IN AN ALL-INSIDE ACL RECONSTRUCTION

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Effective soft-tissue graft fixation to the tibial tunnel in all-inside anterior cruciate ligament (ACL) reconstructions has been reported to be a problem and may lead to retrograde pullout at ultimate load testing.

The purpose of this study was to assess soft-tissue ACL graft load and stiffness when comparing a retrograde bioabsorbable screw fixation with a backup cortical-cancellous suspension suture button to either fixation technique alone in an all-inside ACL tibial tunnel grafting technique. Eighteen porcine tibiae (average bone mineral density of 1.46, measured by DEXA scan), and 18 bovine extensor tendon allografts were divided into three groups of six specimens each before cyclic (500 cycles, 50-250 N, 1 Hz) and load-to-failure (20 mm/min) testing.

Results indicated that in load-to-failure testing, the retrograde screw with suture button combination group withstood significantly higher initial failure (873.87 ± 148.74 N) than did the retrograde screw (558.44 ± 126.33 N; $p < .01$), and suture button only (121.76 ± 40.57 N; $p < 0.0001$) groups. Additionally, ultimate loads were also significantly higher for the combination group (1027 ± 157.11 N) when compared with either the retrograde screw (679.00 ± 109.44 N; $p < 0.01$) or suture button (161.00 ± 29.27 N; $p < 0.0001$) groups.

Soft tissue grafts fixed with a combination of a retrograde screw and a suture button were able to withstand higher initial failure and ultimate failure loads than were the grafts fixed with either a retrograde screw or a suture button alone.

EFFECTS OF PROGESTERONE ON THE ESCALATION OF COCAINE SELF-ADMINISTRATION IN FEMALE RATS SELECTIVELY BRED FOR HIGH AND LOW SACCHARIN INTAKES

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Preclinical studies have shown that progesterone decreases the acquisition, escalation, and reinstatement of cocaine seeking in female rats. Rats bred for low-saccharin (LoS) preference also demonstrate decreased cocaine self-administration relative to high-saccharin (HiS) rats.

The purpose of this study was to examine the cumulative effects of progesterone treatment and saccharin preference on the escalation of cocaine self-administration. Female Wistar rats bred for either HiS or LoS were implanted with catheters in the jugular vein. Daily subcutaneous injections of either vehicle (peanut oil) or progesterone (0.5 mg/kg) were administered throughout the study. Rats were trained to self-administer 0.8 mg/kg cocaine under a fixed-ratio (FR1) schedule during 2-hour daily sessions. Once acquisition criteria were met, rats were allowed to respond for randomly selected cocaine doses (0.2, 0.4, 0.8, 1.6 mg/kg). The cocaine dose was then changed to 0.4 mg/kg, and the session length was extended to 6 hours for 21 days.

Subsequently, cocaine intake under the dose response condition was reassessed. Following cocaine self-administration, saccharin testing was performed on each rat to confirm selective breeding status.

Initial results indicate that during the pre- and post-escalation conditions, HiS female rats received a greater number of infusions compared with their LoS counterparts at the 0.2, 0.4, and 0.8 mg/kg cocaine doses. LoS females reduced their cocaine intake more than did HiS females when treated with progesterone during escalation. The present results suggest that saccharin preference can incur a heightened vulnerability to drug addiction leading to a reduced hormone treatment receptivity.

COMPARISON OF MALE AND FEMALE HOME RANGE SIZE IN MEADOW VOLES (*Microtus pennsylvanicus*)

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There are two basic types of mating systems in the animal kingdom: polygamy and monogamy. Due to research done on home ranges and their connection to mating systems, home range data on a species whose mating system is unknown can be used to predict its mating system (Gaulin and Fitzgerald 1986). Because of the polygynous mating system used by our study species, males were expected to have a larger home range size than females in order to increase the likelihood of encountering a female to mate with.

For our study, we trapped and subsequently observed the spatial movements of individual meadow voles (*Microtus pennsylvanicus*) in the Linnaeus Arboretum of Gustavus Adolphus College over a period of three months to compare the home range sizes of males to those of females. Fluorescent powder and UV lights were used to track the voles' movements 36 hours after capture. Home range size was determined by the minimum convex polygon method, and total distance traveled. We also plotted the ranges using GPS coordinates. We found that males and females had significantly different home range sizes based on total distance traveled (t-test, $p = 0.038$), but no significant difference was found according to the minimum convex polygon method (Rank Sum, $p = 0.170$). Our data supported our hypothesis that males would have a larger home range size than females. We also observed that there were several more females than males present in each study area. Biologically this makes sense, since *M. pennsylvanicus* is a polygynous species.

Source: Gaulin, S. J. C., and R. W. Fitzgerald. 1986. Sex differences in spatial ability: an evolutionary hypothesis and test. *American Naturalist*, **127**, 74-88.

HAPLOTYPE DETERMINATION FOR ISOLATED POPULATIONS OF GIANT REED GRASS (*Phragmites australis*) IN STEARNS COUNTY, MINNESOTA

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Phragmites australis (Cav.) Trin. ex Steud. has been a constituent species of the North American wetland flora for at least 40,000 years. Within the past 150 years a morphologically indistinguishable strain (haplotype M) of this species has quickly invaded wetlands in eastern North America and the Midwest. This invasive haplotype represents a significant risk to wetland ecosystem structure and biodiversity in Minnesota.

In an effort to assess the threat of this non-native haplotype on the aquatic habitats of a 2,000-acre habitat preservation project near Avon, MN, we used PCR-RFLP techniques to analyze chloroplast DNA of recently identified *Phragmites australis* populations in and near the project for haplotype determination. In addition, herbarium specimens dating back to the early twentieth century from a well-documented population (Pleasant Lake) in the region were also analyzed to provide a historical reference for the area.

HOW SEASONS AND WEATHER PATTERNS AFFECT VOCs MEASURED BY MASS SPECTROMETRY

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Volatile organic compounds (VOCs) in the atmosphere can affect ground level pollution as well as a variety of climate processes. The results of the deployment of a glow discharge ion source on a proton transfer MS (PT-MS) for detection of VOCs in air are presented.

Calibrations and tests indicate a good sensitivity, a few HZ PPTV⁻¹ under normal conditions and detection limits for some compounds (1-s integration time) are ~ 100 PPTV. These studies show the ease of use of a glow discharge source for PT-MS studies. The PT-MN ion drift tube has also been improved with internal lenses. The system has proved robust in operation over extended periods of time while being exposed to high and variable concentrations of analytes. With its high sensitivity and utility, the system will prove useful for detection of VOCs in a variety of experiments. This system has been useful in studying human VOCs, sampling outdoor air, and sampling plant-emitted VOCs. For outdoor air, we have observed that weather patterns and seasons have a great effect on levels of various VOCs mostly through wind direction and type of season. This research can be

applied in urban air quality studies as VOCs can be pollutants and can also lead to ozone production in the air.

THE FUEL OF THE FUTURE: BIODIESEL. A NOVEL METHOD AND CATALYST FOR BIODIESEL PRODUCTION

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Biodiesel is a renewable fuel that has great potential to address many of the environmental problems caused by petroleum fuels. The traditional method of producing biodiesel, however, requires expensive feedstocks rendering it too expensive to be economically competitive against petroleum fuels. A novel method, dubbed the Mcgyan Process, has been developed that utilizes inexpensive waste feedstocks. The feedstocks used were virgin soybean oil, waste oil from restaurants, swine tallow: a major waste product in the food industry, steric acid: a free fatty acid that is considered a contaminant in the traditional method, and remarkably acidulated soap stock: a waste product of the traditional process. The Mcgyan Process uses zirconia or titania microspheres as the catalyst. They are thermally and mechanically stable and are able to be packed into a HPLC column that acts as a reaction chamber

The reactor was heated between 300°C and 465°C and the methanol and feedstock were pumped separately into the reactor with 2000-3000 psi. The contact times of the reactants varied from 5.6 to 60 seconds. Hydroxide-modified zirconia, phosphate-modified zirconia, and hydroxide-modified titania were shown to catalyze the reaction of waste feedstocks to biodiesel in high yields. 300 MHz H¹ NMR results showed greater than 90% yields for all feedstocks. The Mcgyan Process is insensitive to FFA and water contamination, has fast reaction rates, and produces little waste. By being able to utilize a wide variety of inexpensive feedstocks, the Mcgyan Process provides a solution to making biodiesel economically competitive with petroleum fuel.

THE ROLE OF *sgd-8* IN THE CONTROL OF OOCYTE MEIOTIC MATURATION IN

Caenorhabditis elegans

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Oocyte meiotic maturation is a highly conserved biological process required for sexual reproduction. In human females, the frequency of meiotic errors increases with maternal age, and these mistakes can lead to miscarriage, infertility, or Down Syndrome. Prior work

has shown that there are many similarities between the control of female meiosis in mammals and in a simple metazoan, the nematode *Caenorhabditis elegans*. For example, in both systems, the stimulatory G alpha subunit regulates oocyte meiotic maturation. In *C. elegans*, the *gsa-1* gene, which encodes the stimulatory G protein alpha subunit, is necessary and sufficient to promote oocyte meiotic maturation.

To further understand how *gsa-1* promotes oocyte meiotic maturation, we conducted a large-scale forward genetic screen to identify mutations that suppress the requirement of *gsa-1* for oocyte meiotic maturation effect. One of the mutations identified, *sgd-8* (suppressor of *gsa-1* maturation defect) is represented by five alleles. Preliminary studies show that *sgd-8* mutants are sensitive to somatic RNAi but resistant to RNAi in the germline. Our hypothesis is that an endogenous RNAi pathway might be involved in regulating oocyte meiotic maturation. To address this hypothesis, we are conducting genetic and phenotypic analysis of *sgd-8* mutants. Using snip-SNP mapping, *sgd-8* was mapped to linkage group III. Currently, we are fine mapping *sgd-8* to an interval for positional cloning. Furthermore, we are conducting extensive phenotypic analysis of *sgd-8* to understand its role in regulating oocyte meiotic maturation. The results of our genetic and phenotypic analyses will be presented at the symposium.

ON THE THERMOCHEMISTRY OF C₄H₈

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The six isomers of C₄H₈ (1-butene, *cis*-2-butene, *trans*-2-butene, isobutylene, cyclobutane, and methylcyclopropane) were analyzed by G3 and CBS-Q compound methods. The relative energies were then tabulated and compared to published experimental values. The calculated relative energies in kJ/mol for G3 and CBS-Q methods, respectively, were found to be isobutylene (0, 0), *trans*-2-butene (5.1, 2.3), *cis*-2-butene (11.3, 10.5), 1-butene (16.5, 15.4), methylcyclopropane (47.3, 43.9) and cyclobutane (52.0, 50.5).

DEMONSTRATION OF REMOTE TEMPERATURE DETERMINATION WITH A NOVEL DUAL-BAND IR IMAGING SPECTROMETER

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A novel dual-band infrared focal plane array has been used to develop a multispectral imaging spectrometer. In addition to perfect spectral registration,

its low mass and compactness make the spectrometer ideal for use in space-based applications. The effective wavebands are 3.75-5.2 and 7.5-10.5 microns. A new method and algorithm for remote temperature sensing that requires only two narrow wavebands of data has also been developed. This spectrometer, with its relatively wide wavebands, presents the opportunity to efficiently investigate the algorithm. Specifically, tests have been made by choosing both widely and narrowly separated wavebands, many and few averaged frames, wider and narrower wavebands, and high and low signal-to-noise ratios. Initial results are encouraging and have demonstrated the utility and limitations of the algorithm. Recovered temperatures have predictable accuracies to within 0.3%, but as large as 50% depending on the parameters chosen.

SYNTHESIS OF PSEUDO-A-TYPE POLYOXOMETALATE KEGGIN SANDWICHES, [M₃(B- α -XW₉O₃₃)₂]ⁿ⁻, AS BUILDING BLOCKS FOR THE PREPARATION OF SUPRAMOLECULES

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In current decades, significant energy has been put into the development and synthesis of unique individual Keggin sandwich polyoxometalate (POM) compounds. These versatile compounds allow for the incorporation of many different redox-active metal cations and have valuable roles in catalysis. The pseudo-A-type POM sandwich [M₃(B- α -XW₉O₃₃)₂]ⁿ⁻ (M = various first-row transition metal cations; X = various main group cations) has an open framework which allows access to the reactive "sandwiched" metals. This experiment radically extends the synthesis of pseudo-A-type POM complexes to form supramolecular nanostructures. The structure is anionic and is valuable as an anionic compound in a field where the preparation of supramolecules has remained largely cationic.

First, the individual POMs, [XW₉O₃₃]⁹⁻, were synthesized with various heteroatoms (X = As³⁺ or Sb³⁺). Then the POMs were allowed to react with Zn²⁺ and Cu²⁺ metal cations to form individual pseudo-A-type Keggin sandwiches. Ligand exchange was performed, replacing the water ligands with biphenyl dicarboxylic acid, forming a lattice structure. Infrared spectroscopy was used to confirm the general structure by comparison with the literature, and single crystal X-ray diffraction was used to more fully examine the structures of the complexes.

EFFECTS OF NITROGEN-TO-PHOSPHORUS RATIO OF NUTRIENT SUPPLY ON PHOSPHATE UPTAKE BY ALGAE AND BACTERIA

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The relative availability of nitrogen and phosphorus in stream water influences algal and bacterial quantity and stoichiometry. These organisms differ in their nutrient storage capacity, leading us to hypothesize a link between storage capacity and N:P stoichiometry of uptake in response to nutrient additions. We investigated the effect of increasing N:P ratio on P uptake in algal and bacterial cultures. To produce algae- or bacteria-dominated biofilms, we used Maracyn tablets, (anti-bacterial), AlgaeFix Pond-Care, (algicide), and diuron (algicide and anti-cyanobacterial). Because bacteria and cyanobacteria are prokaryotes with low nutrient storage capacity, we predicted homeostasis in uptake N:P. Therefore, P uptake was predicted to increase when ambient N:P supply was increased from 5 to 48:1. In contrast, algae are eukaryotes and can store non-limiting nutrients in vacuoles.

We hypothesize that algae draw on PO_4 stores when N supply increases. Therefore, algal P uptake was predicted to change little when N:P increased. In the Maracyn treatment, phosphorus uptake decreased when N:P increased ($13.01 \mu\text{g hr}^{-1}\text{L}^{-1}$ vs. $10.15 \mu\text{g hr}^{-1}\text{L}^{-1}$, respectively) and was greater than in the diuron treatment ($1.27 \mu\text{g hr}^{-1}\text{L}^{-1}$ vs. $0.97 \mu\text{g hr}^{-1}\text{L}^{-1}$), the AlgaeFix treatment ($0.51 \mu\text{g hr}^{-1}\text{L}^{-1}$ vs. $0.97 \mu\text{g hr}^{-1}\text{L}^{-1}$), or the untreated controls ($0.83 \mu\text{g hr}^{-1}\text{L}^{-1}$ vs. $1.02 \mu\text{g hr}^{-1}\text{L}^{-1}$). Increasing N:P of supply did not significantly increase phosphate uptake in the diuron-treated cultures or the untreated controls, but did increase it in the AlgaeFix treatment. These results suggest that the mixed culture of cyanobacteria and bacteria exhibited homeostasis in N:P uptake, while all other treatments exhibited a more plastic response.

CRYSTAL AND MOLECULAR STRUCTURE OF AN UNANTICIPATED DIAMINE BYPRODUCT OF A SCHIFF BASE SYNTHESIS

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We have been investigating the solid-state structures of pairs of isomeric benzylideneanilines differing only in the orientation of the bridge atoms (Ar-CH=N-Ar' vs. Ar-N=CH-Ar') to identify pairs that assume similar molecular packing arrangements in the crystal. Such *isostructural* “bridge-flipped” isomers might be suitable for the formation of solid solutions of the two isomers; the properties of the solids thus formed might be

“tailorable” by the inclusion of systematically varied amounts of the two isomers in the solid. We have focused on pairs of benzylideneanilines in which both isomers bear one halogen atom and one nitrile group to determine whether Lewis acid-base contacts in the solid state of the form $\text{---X-R-C}\equiv\text{N:---X-R-C}\equiv\text{N:---}$ might link molecules into chains in both isomers, with the formation of similar chains favoring isostructuralism.

Of the halogens, iodine is the most likely to act as a Lewis acid in these interactions. In an attempt to prepare *N*-(2-iodobenzylidene)-4-cyanoaniline by the condensation of 2-iodobenzaldehyde with 4-cyanoaniline, we obtained a crystalline product and determined its structure by single-crystal X-ray diffraction. In contrast to all of our previous benzylideneaniline syntheses, this reaction yielded a product in which *two* substituted anilines reacted with one substituted benzaldehyde. The molecule, $\text{I-C}_6\text{H}_4\text{-CH-(NH-C}_6\text{H}_4\text{-C}\equiv\text{N)}_2$, is propeller-shaped, and its packing arrangement involves not nitrile---halogen contacts but nitrile---H-N contacts. A search of the Cambridge Structural Database reveals only seven previous examples of this kind of di-addition product, all involving a limited range of primarily electron-deficient aldehydes (chloral, formaldehyde, or isomeric pyridines).

PRAIRIE RECONSTRUCTION: GERMINATION AND GROWTH OF PLANT SPECIES IN DIFFERENT SOILS

David Manning, David McClure, Paul Roback (Advisor), Kathleen L. Shea (Advisor), and Diane Angell (Advisor)

Center for Interdisciplinary Research

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In recent years, prairie reconstruction projects have become commonplace in many areas that were once primarily agricultural fields. These reconstruction projects provide new habitat for a variety of native prairie species. It is important to know as much as possible about the outcomes of prairie reconstruction projects in order to ensure that a functioning prairie community is established. Recent reconstruction projects at St. Olaf College have yielded varying plant communities despite using similar seed combinations and dispersal techniques.

Here we begin to explore the underlying causes of this variation in order to make future restoration projects more effective. We compared germination and growth of two species of prairie plants (*Amorpha canescens* and *Ratidiba pinnata*) in soils taken from a remnant prairie, a cultivated field, and a restored prairie. Additionally, one set of soil from each soil type was sterilized to determine if rhizosphere differences were responsible for the observed variation. Longitudinal data was collected by introductory ecology classes using a $3 \times 2 \times 2$ factorial design. Factor effects on germination and production were assessed using logistic regression, multiple linear regression, and multi-level models. Results

suggest that microbial influences have a large influence on the germination and growth of both *A. canescens* and *R. pinnata*.

FLUORESCENCE SPECTROSCOPY OF MICROSCOPIC SAMPLES

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We have built a fluorescence microscope with the capability of scanning emission spectra for all objects in the field of view simultaneously. Images are taken using a variable liquid crystal filter (VLCF) that can be rapidly tuned throughout the visible range. Our interest is in quantifying chemicals, such as drugs, in tiny volumes, such as subcellular compartments of a biological cell. These types of samples represent a mixture of microenvironments and present challenges for analysis. Many fluorescent compounds exhibit changes in their spectral profile and their quantum yields depending on the conditions of their surroundings. Spectral images may help circumvent some of the limitations for quantitative analysis of these types of samples by conventional fluorescent microscopy. Examples of images and spectra from a variety of fluorophores, including some anthraquinones, in heterogeneous media are presented here.

WHERE DOES THE ENERGY COME FROM IN ENERGY DRINKS?

Korisa Matezevich, Arlin E. Gyberg (Advisor), and Sue E. Hill (Advisor)

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To investigate the presence of methylxanthine derivatives, specifically theobromine, theophylline and caffeine as compounds in commercial energy drinks.

ABDOMINAL LOADS AND THE COST OF WALKING IN WOMEN

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Increased visceral abdominal fat (VAF) contributes to a positive feedback loop which involves insulin resistance and decreased physical activity and is a risk factor for a number of chronic diseases common in women. The added mass may make walking more slowly necessary for females to offset some of the energetic costs associated with carrying extra mass.

To evaluate this hypothesis, we tested females (N = 20) walking unloaded and front-loaded (two loads, of 8% and 16% of body mass) and at four speeds (self-selected from eight options) centered around optimal walking speeds. We developed cost of transport (CoT) equations from each of the four speeds for each of the three loading conditions for each individual and determined the minimum CoT for each individual at each loading condition. We ran paired *t*-tests to determine that indeed females carrying frontal loads do have a significantly lower optimal walking speed than when they were unloaded ($p < 0.04$). This implies that when traveling a given distance, females with significant VAF would most likely have to walk more slowly than females with less VAF or incur much greater costs. A 16% increase in front-loaded mass incurs a 12% increase in cost with a simultaneous 5% decrease in speed. In real terms, this means that when comparing a female with VAF equivalent to 16% body mass and a female with very little VAF (of the same non-loaded mass) both walking 20 km, the higher VAF female takes 13 minutes longer and uses 127 Cal more than the lower VAF female.

SYNTHESIZING A NOVEL ANTIMICROBIAL AGENT

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In recent years, multi-drug-resistant strains of bacteria have been causing problems for human health. Oxazolidinones, a synthetic class of antibiotics, have proven to successfully combat resistant bacteria. From this class, only one compound, trademarked Zyvox, is on the market today. New synthetic routes are continually being explored to produce more effective antibiotics in this class.

The focus of my research is the synthesis of an oxazolidinone antibiotic functionalized with a thiadiazole group. Originally, a six-step synthesis was laid out for this compound, but new methods have been found to shorten this synthesis. The full synthesis will be discussed, as well as the new routes to more efficiently convert an alcohol to an amine.

SPEED OF MINIMUM COST OF WALKING: AGE AND LOAD-RELATED DIFFERENCES IN WOMEN

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Visceral abdominal fat (VAF) increases with age-independent changes in body mass index (BMI).

Having significant amounts of VAF is a risk factor for certain chronic diseases. VAF increases due to a positive feedback loop involving decreased physical activity and insulin resistance.

To determine possible differences in load-carrying abilities as a function of age, we measured metabolic cost in 10 younger females (mean = 20.9 years) and 10 older females (mean = 51.4 years) as they walked on a treadmill unloaded and front-loaded (two loads, 8% and 16% of body mass) at four speeds (self-selected from eight options) centered around optimal walking speeds. For each individual, we developed cost of transport (CoT) equations (cost vs. speed) for each of the three loading conditions and then determined the minimum CoT (minCoT) and the speed of minCoT (SPminCoT) at each loading condition.

Both age cohorts showed the same pattern of significantly higher minCoT and lower SPminCoT as load increased. Cohorts did not differ significantly (independent *t*-test) in body mass ($p = 0.44$), BMI ($p = 0.93$), stature ($p = 0.13$), lower limb length ($p = 0.21$), or waist circumference ($p = 0.89$). When load level was accounted for (general linear model), we found the two age cohorts did not differ significantly in minCoT ($p = 0.96$), but the SPminCoT showed a statistically significant small drop in the older group ($p < 0.001$). Thus, both cohorts were very similar physically and physiologically and showed a small difference in the speed at which the minimum cost of transport occurs. These results imply that age-related VAF gains could change physical activity patterns.

PHOTOPERIOD ALTERS THE PHASE DIFFERENCE BETWEEN ACTIVITY ONSET *in vivo* AND *mPer2::LUC* PEAK *in vitro*

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Photoperiod is a significant modulator of behavior and physiology for many organisms. In rodents, changes in photoperiod are associated with changes in the period and photic resetting of circadian pacemakers. Utilizing rhythms of *in vivo* behavior and *in vitro* *mPer2::luc* expression, we investigated whether different entrainment photoperiods (16:8 and 8:16 L:D) alter the period or phase relationships between these rhythms and the entraining light cycle. We also tested whether *mPer2::luc* rhythms differed in anterior and posterior SCN slices.

Our results demonstrate that photoperiod significantly changes the timing of the *mPer2::luc* peak relative to the time of light offset and the steady-state activity onset *in vivo*. In both 8:16 and 16:8 L:D, the *mPer2::luc* peak maintained a more stable phase relationship to the end of activity and to light onset, while

dramatically altering the phase relationship to dark onset. Steady-state rhythms of *mPer2::luc* in SCN slices were similar for anterior and posterior slices. Following the initial cycle in culture, the period, phase, and overall rhythm waveform (e.g. peaks per cycle) were not significantly different for anterior or posterior SCN slices. Photoperiod does not significantly alter circadian period of *mPer2::luc* expression *in vitro*, although behavioral studies indicated these periods induced an aftereffect on period *in vivo*. These results suggest that peak of *Per2* expression is linked to activity offset in both anterior and posterior SCN of mice under altered photoperiods.

ALLELOPATHIC FOLIAGE EFFECT OF NATIVE, WOODY PLANT EXTRACTS ON THE GERMINATION, ROOT MASS, AND BIOMASS OF COMMON BUCKTHORN (*Rhamnus cathartica*) IN ST. PAUL, MINNESOTA

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A preliminary sampling of six proximal trees—three sugar maple (*Acer saccharum*) and three silver maple (*Acer saccharinum*)—for buckthorn shrubs along a five-foot radius yielded differing results in mean buckthorn stem length (sugar maple mean = 77.67 cm, silver maple mean = 187.42cm; P value one-tail t-test = 1.22×10^{-9}). Less convincing evidence was found for differing total mass of buckthorn from the same sample (sugar maple mean = 201.5g, silver maple mean = 1960.57g; P value one-tail t-test = 0.051082).

Foliage from six native, woody plant species—sugar maple (*Acer saccharum*), red pine (*Pinus resinosa*), bur oak (*Quercus macrocarpa*), white oak (*Quercus alba*), plains cottonwood (*Populus sargentii*), and common hazel (*Corylus avellana*)—were collected to investigate possible allelopathic foliage effects against the germination, root mass, and biomass of buckthorn. After 45 days of growth, control seedlings appear to have greater germination, emergence, and above-ground mass values. Further seedling growth and data collection will precede presentation of this paper.

ICE-OUT DATE, PRECIPITATION, AND THE START OF THE WALLEYE SPAWNING RUN ON LAKE KORONIS AND RICE LAKE IN STEARNS COUNTY

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Climate change may change spawning dates of fishes, which could then have an impact on the overall ecology of an area. In this study we improved ice-out records for the two lakes by examining the local paper of

the area, *The Paynesville Press*, in the Minnesota Historical Society archives. The dates in question were from 1950 to 2007. Then a complete walleye spawning record was obtained from the local DNR Fisheries Office in Spicer, Minnesota, for the two lakes. The dates for ice-out and the start of spawning run were given a numerical number based on the first day of the year. The residual number was determined by finding the difference between these two dates. Then these residual numbers were compared to the percent increase or percent decrease of early spring precipitation.

EFFECT OF NITROGEN AVAILABILITY ON SPATIAL DISTRIBUTIONS OF CREOSOTEBUSH (*Larrea tridentata*) IN TWO DESERT ECOSYSTEMS

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In many desert plant populations, individuals appear to be uniformly distributed. Creosotebush (*Larrea tridentata*) has been shown to exhibit this uniform distribution; however, little work has been done to determine the factors that lead to this pattern.

We sampled two creosotebush populations in the Mojave Desert and two populations in the Sonoran Desert to confirm distribution patterns and to investigate the possible influence nitrogen availability may have on spacing patterns. We measured height, two diameters, and distance to nearest neighbor for each plant in twenty-five 10 x 10 meter plots at each site in order to determine distribution. The distribution was determined using a dispersion index and statistical significance was determined using a computer simulation. Leaves were collected from each bush to estimate productivity during the previous year. We measured leaf and soil carbon and nitrogen content using an elemental analyzer. Three out of the four sites were significantly more uniform than would be expected by chance alone.

Our findings suggest that as distance to nearest neighbor increases, cover also increases, likely as a result of competition between plants. Average leaf carbon-to-nitrogen ratios were higher in the Mojave Desert than in the Sonoran Desert, indicating higher N availability in the Sonoran Desert. Average productivity was also higher in the Mojave Desert than in the Sonoran, suggesting productivity in creosote is not limited by nitrogen availability. We conclude that creosote are in general uniformly distributed; however, we found little evidence that competition for nitrogen is the factor causing this distribution.

NUMERIC SOLUTIONS FOR THE QUASI-NORMAL MODES OF BLACK HOLES

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Perturbations or quasi-normal modes of black holes are of interest due to their relevance to gravitational wave astronomy. Highly damped quasi-normal modes (QNMs) of black holes have also been conjectured to have an insight into quantum gravity. Specifically, the highly damped QNMs approach a limit suggestive of a relationship between the area and the entropy of the event horizon.

Using a technique developed by Leaver to compute the QNMs for Schwarzschild black holes by developing a characteristic wave equation similar to that of the spectra of a hydrogen molecule ion, and a method of finding multiple roots of a single variable equation as developed by Maehly, we numerically compute a set of QNMs for Schwarzschild black holes for a range of multipole moments. We will extend these calculations to include other types of black holes in order to explore certain regions of the quasi-normal mode spectrum which have not yet been studied.

HOST PLANT VARIATION AFFECTS OVIPOSITION AND SURVIVAL IN AN HERBIVOROUS, STEM-GALLING FLY (*Eurosta solidaginis*)

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Solidago altissima, tall goldenrod, is the host plant of the stem-galling fly *Eurosta solidaginis*, which oviposits in the plant's apical meristem. The ball-shaped gall increases in size as the larva grows inside the gall chamber. Seven clones of tall goldenrod with large numbers of galls formed by *E. solidaginis* were identified in a 5 ha field in Brooklyn Park, MN. In October every plant in each clone was harvested. We determined the basal diameter, mass and diameter of a 5 cm mid-stem segment, stem diameter above and below the gall, gall height, and gall diameter and volume. The galls were opened and examined for the presence of *E. solidaginis* larvae and its hymenopteran parasitoid *Eurytoma gigantea*, and any larvae present were weighed. In the absence of larvae, the presence of frass was indicative of larval death due to unknown causes.

Significant differences occurred among clones for all parameters. We did not find a significant overall relationship between gall diameter or gall volume and larval mass; however, clones with smaller galls also had a

lower percentage of plants with galls. Although basal stem diameter was not correlated with gall diameter, plants with larger stems were more likely to have a gall. The diameter of the stem below and above the gall was independent of gall diameter or gall volume. Percentage of galls in a clone with *E. solidaginis* larvae ranged from 2% to 65%. Percentage of galls in a clone with *E. gigantea* larvae ranged from 0% to 23%.

BRIDGING THE ATLANTIC: FROM FINLAND TO MINNESOTA

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Upon immigrating to the United States around the turn of the twentieth century, the Tuuras and Miss Valiaho, as products of their times and places, carried with them to Minnesota their cultural and historical identities as Finns. As a result, the collective Tuura-Valiaho family (forged by the marriage of Charles Tuura and Impi Valiaho) has perpetuated many manifestations, such as the rejection of a socialistic past and the implicit understanding of coffee as a social lubricant. However, they did not merely replicate their cultural and historical identities by simply imprinting them upon their descendents. Instead, the collective Tuura-Valiaho Finnish identity has settled both implicitly and explicitly into the lives of their descendents who continue to both affirm and transform their identities as the progeny of Finnish immigrants. This is because the present is essentially a product of the past. Many aspects of the Tuura-Valiaho cultural and historical identity remain intact; however, this identity exists as a dynamic entity that has settled into the lives of dozens of Tuura-Valiahos.

BODY SIZE, GROWTH, AND REPRODUCTION IN THE HERMAPHRODITIC SNAIL *Helisoma trivolvis*

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In hermaphroditic species such as the snail *Helisoma trivolvis*, individuals must allocate their reproductive effort to female and/or male function. In addition, these snails must also allocate available resources between reproduction and other energetically costly activities such as growth, maintenance, and food acquisition. Energy put into reproduction is not available for growth or other activities, a phenomenon often referred to as the “cost of reproduction.”

We investigated the relationship between these two important functions—reproduction and growth—by measuring the body size of snails from hatching until reproductive maturity and then comparing growth in reproductive and non-reproductive (isolated) individuals.

To determine how best to deliver food to the snails, we mixed different amounts of romaine lettuce with agar at 13%, 23%, 35%, and 51%, and then measured the growth of snails fed these different foods. Since growth was dependent upon the percentage of lettuce in the food, and always lower than that of snails fed plain lettuce, we fed the experimental snails plain lettuce. Mated snails laid an average of 23 egg masses in 3 weeks with an average of 19 eggs per mass, and grew more slowly than did the unmated snails during this time. These results support the idea that reproduction is energetically costly and may result in a trade-off with growth.

METALLOTHIONEIN AS A BIOINDICATOR OF FRESHWATER METAL POLLUTION

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Heavy metals (mercury, cadmium) are among the most dangerous substances present in almost all environments. Minnesota lakes and rivers often are posted with signs warning the general populace, especially pregnant women, against eating fish caught in these waters more than once a week. The recent increase in immigrant populations who routinely use fish from these waters for their protein sources has made it imperative that the environmental health of our local waterways be monitored and examined for potential life-threatening concentrations of heavy metals.

The aim of this research is to evaluate the metallothionein proteins from local freshwater fish (i.e. carp, panfish, or river catfish) as bioindicators for the presence of heavy metals in local bodies of water (i.e. Mississippi River and Lake Minnetonka). Metallothioneins (MT) are metalloproteins of low molecular weight that contain repeated amino acid sequences of Cysteine-Xaa-Cysteine or Cysteine-Xaa-Xaa-Cysteine through which mono- or divalent d^{10} metal ions are bound as polymetallic-thiolate clusters. The metals that fall in this category are zinc[Zn(II)], cadmium[Cd(II)], copper[Cu(I)], and mercury[Hg(II)]. These proteins occur normally in the liver and kidneys of higher organisms and their role appears to be the regulation of needed zinc and copper ions. In reviewing the binding affinities of these metals a 10,000-fold higher affinity is seen for Cd(II) over Zn(II), which is the reason for the environmentally less abundant Cd(II) to be accumulated in MT. In river environments in Northern Spain and Brazil, where high levels of these metals have been found, the levels of MTs in eel and brown trout species have been elevated over those of clean water environments.

We hope to repeat the same experimental processes reported in the literature with common fish species caught in Minnesota waters. The advantage of this approach is that it is relatively simple while being

specific, involving minimal processing and using generally available instrumentation. We expect to further purify and characterize the protein we isolate to demonstrate more definitively the nature of the MT protein.

SEMAPHORIN PROTEIN EXPRESSION IN MOUSE THYMUS

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Semaphorins are a family of proteins that were identified as guidance cues during nervous system development. Recently, their role in the immune system has also begun to be studied. It has been found that semaphorins can play a physiological role in the activation and migration of several classes of immune cells. Thus, we hypothesized that they may also guide thymocytes through the thymus. We have evidence for gene expression of Sema4A, 4D, 7A, and their receptors in the thymus of 6-8-week-old mice, using RT-PCR.

We are currently using IHC to study the expression of semaphorin proteins and their localization in specific thymic subsets. Using FC, we found that Sema4D was highly expressed in CD45⁺, but weakly expressed in CD45⁻ cells. In addition, only a small subset of both CD45⁺ and CD45⁻ cells expressed Sema4A. We have also found out, using IHC, that Sema4A is preferentially expressed in the thymic medulla compared with the thymic cortex. We will continue using these methods on C57 mouse thymus to obtain a more complete picture of the distribution of semaphorins and their receptors in the thymus. This distribution pattern can provide starting points for the development of hypotheses about the role of semaphorins in T cell development.

INCIDENCE OF TYPE 1 DIABETES (T1D) IN THE COLONY OF NOD/LtJ MICE AT ST. CLOUD STATE UNIVERSITY (SCSU)

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Type 1 diabetes (T1D) is an autoimmune disease that is characterized by an absence of insulin, because insulin-producing pancreatic beta cells are destroyed by one's own immune T cells. The best animal model for the study of T1D is a NOD mouse model. While the NOD mice are ~99% genetically identical, it has been known that the environmental factors influence the incidence of the T1D. Therefore, it is necessary to study the diabetes incidence in NOD mice in a particular environment before

the further research is performed in these mice. Insulinitis is a characteristic histopathological lesion of T1D that represents an accumulation of the immune cells (mostly T cells and macrophages) in the pancreatic islets of Langerhans

The objective of this research is to study diabetes incidence and kinetics of insulinitis development in the colony of NOD/LtJ mice at SCSU. Two breeding pairs of NOD/LtJ mice were purchased at The Jackson Laboratory, Bar Harbor, ME. The blood glucose levels of female (n = 25) and male (n = 14) mice were measured every second week from 6 to 30 weeks of age. While 4%, 20%, and 68% of NOD females became diabetic at 8, 15, and 30 weeks of age, respectively, 0% of NOD males developed diabetes until 30 weeks of age. The pancreata from female and male NOD/LtJ mice (n = 3-4 mice/time point; 4-, 6-, 8-, and 12-week-of-age time points) were removed, fixed, embedded in paraffin, cut, and slides were prepared for the hematoxylin-eosin staining. The slides will be graded semi-quantitatively for the insulinitis level.

THE CHARACTERIZATION AND COMPARISON OF THE iNOS GENE IN EXERCISE-INDUCED ASTHMA AND ASTHMA-FREE INDIVIDUALS

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Asthma is a chronic disease that is characterized by an increase in bronchial constriction and mucus hypersecretion in the bronchial walls. A variety of stimuli, including direct and indirect, respond to asthma and lead to multiple symptoms. Symptoms such as coughing, wheezing, and shortness of breath can occur. It has been reported that airway-derived nitric oxide (NO) may contribute an important role in inducing relaxation of smooth muscle in the bronchi. Nitric oxide is synthesized by the enzyme nitric oxide synthase (NOS), of which different isoforms, such as iNOS, have been proven to generate small amounts of bronchoprotective nitric oxide in response to certain stimuli to counteract the symptoms.

A recent study has shown an association with the iNOS gene and severity of asthma and eosinophils. The gene for iNOS lies in the CC chemokine cluster region, on chromosome 17. The relationship between this iNOS gene and asthma generated interest to begin an investigation into their association. An Acrylamide Gel Electrophoresis revealed different size bands between samples collected from exercise-induced asthmatics and asthma-free individuals. The study is currently underway, with plans to perform SDS-PAGE on the PCR-prepared DNA samples, as well as further characterization and cloning.

THE EFFECTS OF THE HORMONE LEPTIN ON OBESITY, METABOLISM, BODY TEMPERATURE, AND FEEDING BEHAVIORS, USING NORMAL, OB/OB, AND DB/DB MUTANT MICE

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Why is obesity or being overweight such a battle for some people and not others? A way to address this question is to look at how the body utilizes the hormone leptin, which is derived primarily from adipocytes. Leptin, the product of the obese (*ob/ob*) gene, plays a major role in the regulation of food intake, hunger, satiety, and metabolism.

The purpose of this experiment was to determine if the lack of leptin or the leptin receptor in mutant mice affects basal metabolism, body temperature, and feeding behaviors. Eighteen mice were used in this experiment: 6 controls, 6 mutant *ob/ob* (defective for the expression of leptin), and 6 mutant *db/db* (defective for the expression of the leptin receptor). The variables tested included basal metabolic rate, food intake, water intake, cage waste, and body temperature.

Results demonstrated that normal mice had a statistically higher metabolic rate and body temperature than both the *ob/ob* and *db/db* mice. Although the normal mice ate more than the other mice, the increase was not statistically significant. The water intake was statistically higher in the *db/db* mice compared to the *ob/ob* and control mice. The cage waste was statistically higher in the *db/db* mice (from high urine output) and in the control mice (with large amount of food debris) when compared to the *ob/ob* mice. These results substantiate that mutations in the genetic expression of leptin or the leptin receptor may contribute to interruptions in normal metabolism and may contribute to obesity.

DEVELOPMENT OF A NOVEL ASSAY AND PURIFICATION PROCEDURE FOR NITROBENZENE DIOXYGENASE

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Nitroaromatic compounds are rare in nature, but are used in industry for the production of dyes, pesticides, and explosives. The bacterium *Comamonas* sp. JS 765 can utilize nitrobenzene as a sole carbon and nitrogen source because it contains a nitrobenzene dioxygenase system (NBDOS) that initiates its breakdown. NBDOS is a member of the Rieske non-heme iron dioxygenase family, a group of multi-component enzymes that catalyze the stereo- and regiospecific addition of diatomic oxygen into aromatic substrates. Formation of *cis*-dihydroxylated

metabolites in this way is an important first step in the degradation of aromatic compounds by bacteria.

The objectives of this project are to (1) develop an assay for measuring NBDOS activity and (2) purify the components of this system to homogeneity for eventual mechanistic studies. Results of the assay and purification will be presented.

IMMUNOFLUORESCENT ANALYSIS OF ACTIN EXPRESSION IN GENETICALLY MODIFIED VS. WILDTYPE MURINE OCULAR MUSCLE

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Actin is a cytoplasmic filament that is present in all eukaryotic cells and contributes to cell shape, cell mobility, and the organization of certain tissues such as striated muscle. Actin is regulated by specialized accessory proteins including actin capping protein (CP) that serves to stabilize the molecule and regulate its length. CP consists of two distinct protein subunits, an alpha (α) and a beta (β). Two isoforms of the β ($\beta 1$ and $\beta 2$) subunit have been identified in vertebrates. Genetically modified mice, engineered by Dr. Hart to express lower levels of $\beta 1$ in their ocular muscles, lost their ability to open their eyes.

We hypothesized that reduced function in the ocular muscles is caused by disruption of actin organization in myofibrils. Characterization of this disruption is key to understanding how reduced levels of $\beta 1$ affect actin organization, myofibrils, and the cell as a whole. Samples of ocular muscle from both wildtype and genetically modified mice were collected and frozen in liquid nitrogen. Tissue sections, 7 microns thick, were prepared using a Leica cryomicrotome, probed with a fluorescent actin antibody, and visualized using an Olympus fluorescent microscope. Digitized images were acquired using Simple PCI software.

DOES WHI-P131 INDUCE APOPTOTIC CELL DEATH IN THE ISOLATED CD4+ T CELLS OF NOD MICE?

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Janus tyrosine kinase (JAK) 3 is a signal transduction molecule that is expressed by T cells. By inhibiting JAK 3, it might be possible to disrupt the function of autoimmune T cells and thereby prevent development of autoimmune diseases such as type 1 diabetes (T1D). It has been shown that WHI-P131, a specific JAK3 inhibitor, can prevent T1D in a mouse

model (NOD mouse) of T1D. The mechanism of WHI-P131 action is not known.

The goal of this research is to study whether WHI-P131 induces cell death in T cells exposed to the drug *in vitro*. The cell death was studied in the heterogeneous population of splenocytes, as well as in isolated CD4⁺ T cells of 4-7-week-old NOD and control C57BL/6 female mice. Cells were cultured in a 96-well-plate, stimulated with either mitogen or by anti-CD3 plus anti-CD28 antibodies for a time period of 2, 7 and 14 days. WHI-P131 was added in the concentrations of 6, 3, and 1.5 $\mu\text{g/ml}$. Cell death was measured by Cell death detection ELISA (Roche Diagnostics). The dose-response-dependent induction of apoptotic cell death was observed by addition of the drug in both heterogeneous, as well as in the isolated CD4⁺ populations of T cells. The absorbance of the cells (both entire splenocytes and isolated CD4⁺ T cells) exposed to the highest concentration of WHI-P131 (6 mg/ml) was four to seven times higher compared with the absorbance of control cells ($p < 0.05$). These results suggest that WHI-P131 induces apoptotic cell death in cultured CD4⁺ T cells.

RAP'S ROLE IN THE PI3K SIGNALING PATHWAY

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Rap is a small G-protein that is closely related to Ras, which has been shown to play a role in human cancers. However, many of the functions of Rap in the cell are unknown. Previous research in our lab has shown that Rap has an effect on proliferation, migration, and the activity of the protein kinases Akt and Jnk. The literature suggests that all of these effects may be linked to the phosphatidylinositol 3-kinase (PI3K) signaling pathway. Akt activity in particular is usually directly correlated with that of PI3K.

To study Rap's role in this pathway, cells were transfected transiently or stably with an active Rap1 gene, a control plasmid, and either a dominant-negative form of Rap1 or a Rap-Gap. Western blotting results suggest that Rap's influence over PI3K signaling is cell-type specific. Changes found in active Akt and PTEN levels suggest that Rap inhibits the PI3K pathway in epithelial cells, while activating it in fibroblasts. Flow cytometry for intracellular staining is currently being used to confirm western blotting results and to further characterize the response to active Rap1.

POVERTY AND ITS RELATIONSHIP TO SCHOOL ACHIEVEMENT

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It has been more than three years since No Child Left Behind legislation became a standard in academic policy nationwide. This influential and yet controversial act has had major impact on students in poverty and their academic achievements. This has led me to analyze the relationship of poverty on student achievement. Charles V. Willie states in his publication, "The Contextual Effects of Socioeconomic Status on Student Achievement Test Scores by Race," that there is a direct correlation between what he calls "affluently concentrated schools" and higher test results on standardized tests. In addition, Leventhal, Brooks-Gunn (2004) have shown through their study, "A Randomized Study of Neighborhood Effects on Low Income Children's Educational Outcomes," that as children and their families moved from a high-poverty neighborhood to a low-poverty neighborhood, their test scores improved.

My paper will examine the relationship between poverty and student achievement using the Minnesota Basic Skills Test data. These data include math and reading test scores of all eighth graders in Minnesota public schools. The independent variables will include vouchers for free and reduced lunches as a proxy for income, race, and teacher salary. My hypothesis states that there is a negative relationship between poverty levels and their corresponding test scores.

THE EFFECT OF DIFFERING PHOTOPERIODS ON THE OUTPUT GENES, *PSBA1*, AND *PURF* OF THE CYANOBACTERIUM *Synechococcus elongatus*

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Cyanobacteria are single-celled prokaryotes that exist and thrive in most habitats on earth and use a circadian clock to efficiently regulate their cellular activity. The oscillator genes *kaiA*, *kaiB*, and *kaiC* are responsible for the circadian mechanism and therefore regulate output gene activity. Genes such as *psbA1* (important for photosynthesis) and *purF* (important for purine biosynthesis) are examples of two output genes that are expressed in the cyanobacterium *Synechococcus elongatus* PCC 7942 due to the circadian clock.

Photoperiods have been shown to have considerable impacts on circadian clocks in other model systems; however, the effect of photoperiod on the cyanobacterium *Synechococcus elongatus* is not well understood. To determine the effects of photoperiod on cellular activities, *S. elongatus* reporter strains AMC408 (*purF::luxAB*), AMC669 (*psbA1::luxAB*), and the wild-type AMC462 were exposed to 12L:12D (12 hours of light and 12 hours of dark), 6L:18D and 18L:6D photoperiods. The wild-type *S. elongatus* results showed a 4-hour phase delay during the 18L:6D photoperiod and a 4-hour phase advance in the 6L:18D photoperiod when

compared to the 12L:12D phenotype. Preliminary results suggest that the AMC408 strain showed little difference in circadian oscillation in response to the different photoperiods, while AMC669 demonstrated a phase advance when exposed to either an 18L:6D or 6L:18D photoperiod compared to 12L:12D. These preliminary results suggest that the different photoperiods differentially affect the circadian expression of the wild type *S. elongatus* and the different *S. elongatus* output genes, *purF* and *psbA1*.

SEQUENCING AND EXPRESSION OF THE *FGF-10* GENE IN *Xenopus laevis*

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During mammalian lung and limb development, interactions between the epithelial and mesenchymal layers govern the branching of the lung and limb, respectively. The Fibroblast Growth Factor (FGF) gene family has been shown to play an intricate role in the governing of these interactions. The *FGF-10* gene, in particular, has been shown to play an essential role in these communications, and its deficiency results in the inability for the lung to branch out from its initial bud in mice.

The *FGF-10* gene was isolated from *Xenopus laevis*, and its entire open reading frame sequence was able to be obtained using fragments of the sequence from previous Bethel University student research. In addition, expression patterns of the *FGF-10* gene were analyzed in various stages of *X. laevis* development.

EFFECTS OF PRAIRIE AGE AND VEGETATION STRUCTURE ON SMALL RODENTS IN MINNESOTA PRAIRIES

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Tall-grass prairies are important as sources of food and habitat for plants, animals, and humans; however, less than 1% of the Midwest's historic tall-grass prairies remain today. This decline in prairies has been accompanied by a reduction in prairie-dependant small rodents. Although people have actively started recreating prairies, it is not clear that these reconstructed prairies support populations of prairie rodents.

Through live trapping, this study found that there is little correlation between the age of reconstructed prairie sites and the number of small rodent individuals and species. In Carleton College's Cowling Arboretum it was also found that more rodent species occupy prairie sites with higher numbers of plant species. This trend was not found in St. Olaf College's Natural Lands, however,

possibly due to a lower overall number and quality of plant species in the prairie sites. A rodent survey of reconstructed and remnant prairies in and around Rice County, MN, found two sites with prairie voles, a Minnesota species of special concern. This suggests that prairie voles can and do still occupy prairies in the area.

HOW SPECIES-SPECIFIC IS AN OBLIGATE MUTUALISM?

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Pollinators can lie anywhere on a continuum between total generalist and total specialist. Until recent findings in the Neotropics, fig wasps (Agaonidae) and the plants with which they have an obligate mutualistic pollination relationship (*Ficus*, Moraceae) were thought to be at the extreme specialist end of this continuum.

This study used mitochondrial DNA sequences to identify pollinators to see if they visit one or multiple hosts. Pollinator sharing was witnessed at a very low frequency (<1%) and pollinators for a putative hybrid species of *Ficus* had enough genetic divergence to form two distinct clades in a phylogenetic tree.

COMPARISON OF LEAD CONTENT IN WATER SOURCES COLLECTED IN THE TWIN CITIES

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Lead is a toxic metal that has harmful health effects on the human body. The effects are most severe for infants and children who, when exposed to lead, can experience delays or suspensions of physical and mental development. In adults, exposure to lead can cause kidney problems or high blood pressure. A common source of lead exposure comes from drinking tap water. Therefore, the use of lead components in plumbing systems has been prohibited. According to the Environmental Protection Agency, the simplest way to decrease lead content in systems constructed prior to the enactment of these regulations is by flushing pipes thoroughly before using the water.

This project examines lead content in tap water samples collected before and after flushing. Testing of lead in imported bottled water and samples collected from other sources was conducted using the Buck Scientific 210/211 VGP Atomic Absorption Spectrophotometer. The results will be compared with the standard considered safe according to the Environmental Protection Agency

COMPARING THE MARCOINVERTEBRATE COMMUNITIES OF TWO PRAIRIE POTHOLES WITH VARYING WATER QUALITY DURING THE WINTER SEASON

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During the winter of 2007 we sampled the benthic macroinvertebrate community in a eutrophic prairie pothole, Page Lake, in Stevens County, MN, to determine the sample size necessary to obtain a meaningful estimate of the community diversity and abundance in the profundal zone. Our results indicated that a minimum of 10 samples was required. We are currently sampling the profundal zone of a nearby pothole, Cottonwood Lake in Grant County, which has undergone multiple watershed improvements. We selected sample sites randomly along transects starting 50m from shore and sampled by lowering an Ekman bottom grab sampler through the ice, collecting 0.02 m² of sediment and associated organisms. Samples were washed through a 500-micron mesh. Macroinvertebrates retained by the mesh were picked from the sediment, preserved in 80% ethanol, and identified to the lowest feasible taxonomic level. Dipteran larvae, water mites, and oligochaetes were common in Page Lake. Diptera and water mites are also common in Cottonwood Lake. Amphipods and Coenagrionidae larvae occur only in Cottonwood Lake.

We analyzed Cottonwood Lake samples to verify if 10 samples will adequately characterize the profundal community. Results from Cottonwood will be used in conjunction with results from Page to provide a template for determining the sample size required to characterize accurately the macroinvertebrate communities in Minnesota prairie pothole lakes. Sampling the macroinvertebrate community during the winter may provide an additional opportunity to evaluate how surrounding land use affects aquatic communities.

INVESTIGATING INTERACTIONS OF PROTEINS ON METAL SURFACES

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The investigation of protein interactions on different metal surfaces is of interest for a variety of reasons, which include gaining basic knowledge about how proteins adhere to certain surfaces, and also some medical field applications. The protein that is being studied is insulin, and the metal surface currently being used is stainless steel. A matrix-assisted laser desorption/ionization (MALDI) technique is the main source of analysis for this

investigation. The MALDI target is the source of the metal surface. The volume of the protein is kept constant, along with the volume of the matrix. This experiment is focusing on how different concentrations of insulin will affect its interactions with a surface. While insulin is kept constant, along with the matrix, the metal surface is changed.

INFERRING SOCIAL HIERARCHIES BASED ON A QUANTITATIVE COMPARISON OF INDIVIDUAL BEHAVIOR IN A CAPTIVE TROOP OF *Macaca fuscata*

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Japanese macaques (*Macaca fuscata*), also known as Japanese snow monkeys, are primates that are endemic to Japan. The Minnesota Zoological Gardens has been home to a troop of Japanese macaques since its opening in 1978. Macaque troops are characterized by a strict social hierarchy among both males and females. Knowledge of these hierarchies in zoo populations can be useful when introducing new reproductive individuals to troops.

Here we constructed a social hierarchy based on quantitative comparisons of behaviors during the mating season. Using the number of mating activities as well as interactions such as positive/dominant interactions and negative/submissive interactions, it was clear that 1506 was the alpha male of the troop and 726 had been reduced to the role of beta male. Using the same interactions as well as time spent in mutual grooming, self grooming and time spent in proximity, the social structure of the females was determined. It appears from this study that 1030 is the alpha female and 805, previously thought to be alpha, is now the beta female. It was also seen that higher ranking macaques tended to be younger than lower ranking macaques.

QUANTIFICATION OF INTERLEUKIN 23 IN HUMAN PROSTATE CANCER

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Many types of human epithelial cancers arise in a background of chronic inflammation. Infiltrating inflammatory cells may damage epithelium leading to chronic reparative cell turnover, release oxygen free radicals causing DNA damage, degrade basement membranes, and trigger microvessel formation that may

enable cancer cells to enter the bloodstream during metastasis.

Using RNA expression microarray analysis, we have found that interleukin 23 (IL23) expression correlates with poor outcome following prostatectomy in prostate cancer patients. IL23 promotes the development of T-helper 17 (Th17) cells, a recently identified subset of Th cells that appears to mediate chronic inflammation.

We hypothesize that IL23 drives chronic inflammation in prostate cancer leading to metastatic disease. Using a cohort of prostate tumor samples from patients with variable clinical outcome, we are addressing the hypotheses that (1) the degree of chronic inflammation is correlated with poor prognosis in prostate cancer, and that (2) the number of IL23-expressing cells correlates with both the degree of chronic inflammation and poor prognosis. We are determining the amount of chronic inflammatory infiltrate present in the prostate tumor samples. And we are using PCR methods to clone a portion of the IL23 gene into a vector suitable for *in vitro* synthesis of radiolabeled RNA probes for *in situ* hybridization studies to quantify IL23-expressing cells in prostate cancer specimens. Results of our study may provide insight into the role of chronic inflammation in prostate cancer biology, and may indicate a potential use for anti-inflammatory drugs being developed to block IL-23 function.

AN ANGIOBLASTIC MECHANISM FOR HEPATIC VASCULOGENESIS?

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Vasculogenesis (blood vessel formation) of embryonic organs occurs by two mechanisms: angiogenic vasculogenesis involves the growth and migration of differentiated vascular endothelial cells (VECs) from pre-existing vessels; angioblastic vasculogenesis instead provides VECs through differentiation *in situ* of the organ's own mesenchyme (Sherer, 1991). The distribution of these mechanisms is determined by their anatomic origin: organs derived from somatopleure (muscle and skin) vascularize angiogenically while those of splanchnic origin (most visceral organs) vascularize angioblastically (Pardanaud et al., 1989). However, the mechanism underlying the formation of the liver's sinusoids remains unclear. Evidence has been published which favors both their (angiogenic) sprouting from the ductus venosus and other vessels (Kingsbury et al., 1956) and their (angioblastic) differentiation from the liver's own mesenchyme (Sherer et al., 1983, 1984).

We are reinvestigating this question by assessing the organization and distribution of vascular endothelial cells in the hepatic region and their proximity to larger, adjacent vessels from which they might sprout. Japanese

quail (*Coturnix coturnix japonica*) embryos at Hamburger-Hamilton stage 14 were fixed in glyoxal, processed into paraffin, and sectioned at 5 microns. Sections of the hepatic region were stained with endothelium-specific QH-1 monoclonal antibody and an Alexa-Fluor 488-conjugated secondary antibody, coverslipped with a DAPI-containing mounting medium, photographed in B&W under epifluorescence, and electronically pseudocolored. Slides were then re-stained with H&E and photographed under brightfield. These photographs are being examined for the presence of QH-1-positive cells in locations remote from established vessels, evidence that we would interpret as confirmation of our angioblastic hypothesis.

INTERACTIONS OF PHOSPHOLIPIDS WITH CYCLODEXTRIN

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A living cell is surrounded by a cell membrane, which consists of a lipid bilayer. This bilayer is a complicated mixture of molecules, each with a polar head and a non-polar tail. Interactions between lipid components are thought to be important, but are difficult to measure directly. Lipids don't naturally mix because their non-polar tails repel water and therefore do not want to leave the cell membrane. When bound to cyclodextrin, however, these lipids are protected from the water and can move freely out of the membrane. Consequently, we use cyclodextrin to catalyze the mixing of these lipids in solution.

In this study, we examine the effect of cyclodextrin on the mixing of lipids with different head groups and non-polar chains. Using an isothermal titration calorimeter, we monitor the minute quantity of heat absorbed or released during the mixing of our lipids. Experiments have shown that as little as 0.1 mM cyclodextrin can be used to effectively catalyze lipid mixing. We have replicated previous results which show exothermic trends during lipid mixing. We will continue with monitoring the mixing of new lipids that differ in non-polar chains and others that vary in head groups.

COMPARISON OF VARIOUS POLARIMETRIC RADAR RAIN RATE ESTIMATORS WITH SURFACE-OBSERVED RAIN GAUGE RAIN RATES

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Two methods are used currently in operational meteorology to estimate rainfall rates. The first method is to use rain gauge instrumentation to measure the amount

of rain that has fallen over a particular amount of time. The second method involves using radar reflectivity in a mathematical relationship to estimate rainfall rates.

This project explores both of these methods while using rain gauge observations as ground truth to compare with radar estimated rainfall rates. This project uses radar data from the Colorado State University CHILL (University of Chicago and Illinois State Water Survey) radar, which has unique, dual-polarization capabilities that yield multiple parameters that can be used to estimate rainfall rates. This project will investigate the accuracy of conventional reflectivity as well as polarimetric rainfall estimation methods for a range of rainfall rates.

The goal of the research project is to determine which estimation methods are most accurate for a given range of rainfall rates. Previous studies have shown that conventional reflectivity estimations can underestimate rainfall rates when very small water droplets are present, and they can under- or overestimate rainfall rates when mixed precipitation is present. This is the reason for studying conventional, as well as polarimetric, parameters when estimating rain rates. The hypothesis is that the estimation methods using polarimetric parameters will perform better than the conventional reflectivity methods because polarimetric parameters can better account for raindrop sizes and shapes. Results from multiple summer precipitation case studies from 2003, 2005, 2006, and 2007 will be discussed.

THE EFFECTS OF SILENCING THE CALCIUM-DEPENDENT PROTEIN KINASE (CDPK1)-BINDING PROTEIN IN *Medicago truncatula*

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A calcium-dependent protein kinase (*CDPK1*) was previously discovered in *Medicago truncatula*. When the *CDPK1* was silenced, the result was shorter root hairs, shorter root cell lengths, and a reduction in symbiosis with both rhizobial bacteria and mycorrhizal fungus. A yeast two-hybrid screen was used with a cDNA prey library to identify *CDPK1*-protein interactions. One protein found was termed the *CDPK1*-binding protein1, or *CDPK1*-BP1. This protein was found to be related to BTB domains, which are thought to interact with cullins in the ubiquitin-dependent protein degradation pathway. It was thought that *CDPK1*-BP1 might work in that pathway. The gene coding for the *CDPK1*-BP1 protein was silenced using RNA interference (RNAi) via *Agrobacterium*-mediated transformation. *M. truncatula* plants with *CDPK1*-BP1 silenced were assayed, with no obvious phenotypic differences found between *CDPK1*-BP1-silenced plants and control plants.

UNIFYING STOCHASTIC SIMULATION OF BIOCHEMISTRY

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For a variety of cellular processes—from metabolic pathways to gene regulation—biologists have developed a fine-grained understanding of the molecular mechanisms involved. Often this knowledge is presented succinctly in the form of a coupled set of chemical reactions. And yet, in spite of the fact that such precise models exist, methods for the quantitative analysis of biological systems remain elusive. Translating a *descriptive* set of reactions into a *computational* framework has proved challenging.

Stochastic simulation has the potential to become a computational workhorse for biology. First proposed by Gillespie, the so-called *mesoscopic* framework for chemical kinetics assumes that a spatially homogeneous mixture of reactants behaves as a discrete stochastic process. The simulation tracks integer quantities of the molecular species, executing reactions at random based on propensity calculations. Repeated trials are performed to characterize the evolution of the system. This framework captures individual molecule dynamics in a way that is impossible with differential equation systems, thus modeling more accurately the real chemistry. The drawback of this approach is the prohibitive computation time that is required. In order to obtain an accurate estimate, a large number of trials must be performed, each trial consisting of a long chain of reaction events. A separate random number must be generated for each event—a costly step from a computational standpoint.

Our research focuses on attacking the computational challenges through new algorithms and through a unified framework for leveraging distributed and high-performance computing resources.

DETECTION OF T CELLS BY IMMUNOFLUORESCENCE

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T cells are white blood cells divided into three subpopulations: T helper, T cytotoxic, and T regulatory cells that express specific markers on their cell surface. The CD4 and CD8 molecules are the markers of the helper and cytotoxic T cells, respectively. The T regulatory cells have both CD4 and CD25 markers expressed on their surface. Based on the expression of the CD molecules, T cells can be detected and quantified by using fluorochrome-labeled antibodies that bind to these markers. The fluorescence can be detected either by

flow cytometer (faster and easier method) or by immunofluorescent microscope (labor-intensive method).

As we do not have a flow cytometer at SCSU, but need to immunophenotype T cells, the goal of this project is to optimize the condition for detection of different T cell phenotypes by immunofluorescence. The immunofluorescent microscope (Olympus B071), which contains a filter for detection of fluorescein isothiocyanate (FITC)-induced fluorescence, was used. The FITC-labeled antibodies (CD4, CD8, CD25) were purchased from the BD Biosciences and Miltenyi Biotec. The (1) freshly prepared and (2) cultured (with addition of either mitogen or anti-CD3 plus anti-CD28 antibodies) heterogeneous T cells and isolated CD4+ T cells (isolated by magnetic anti-CD4-labeled microbeads, Miltenyi Biotec) were obtained from the spleens of 5-8-week-old C57BL/6 and NOD mice. Various concentrations of cells (1-10 X 10⁵/ml), cell fixatives (paraformaldehyde and acetone), slide types (plain and poly-L-lysinecoated), antibody dilutions (1:10-1:100), and antibody types (different anti-CD4 antibodies), tested for visualization of desirable subpopulations of T cells by immunofluorescence, will be discussed.

BIOMASS PRODUCTION AND SOIL NUTRIENT ANALYSIS IN RESTORED PRAIRIES

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Variation in prairie biomass production with species composition and age of prairie was examined in several restored prairies on the St. Olaf College campus in Northfield, MN. The prairies were established from 1980-2004 on land previously used for agriculture and have been maintained through periodic fire disturbances since restoration.

We compared variation in soil nutrient levels, percent moisture, percent organic content, and biomass content. Nitrogen levels showed little variation among prairies, but were significantly lower than in an adjacent agricultural field. Orthophosphate phosphorus showed more variation and ranged from 26 ppm in the 1989 prairie to 83 ppm in the 1998 prairie. Percent soil organic content did not differ significantly, but ranged from 2.988% in the 1989 prairie to 7.5% in the 2001 prairie. Biomass was harvested in 0.25-m² plots and analyzed for grass, forb, and total biomass production. The most recently planted prairie, restored in 2004, had not been burned and showed the highest biomass production of 1673.37g/m². The lowest biomass production of 573.12 g/m² occurred in the 1989 prairie, which was burned in spring 2007. Interestingly, the data suggest that total biomass decreases in older and more recently burned prairies. The prairie restored in 2004 produced the most biomass and also had the highest grass-forb ratio. This indicates that species composition may play an important

role in the amount of biomass produced. Our results suggest that a mixed grass-forb prairie used as an energy source such as cellulosic ethanol will be most productive if harvested once every several years and not annually.

SYNTHESIS AND CHARACTERIZATION OF A NOVEL POLYMERIZABLE CHEMILUMINESCENT COMPOUND

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The synthetic route and methodology toward a new 3-aminophthalhydrazide (luminol) derivative is reported. 4-nitrophthalic acid was converted to a dimethyl ester before reducing the nitro moiety to an amine. A double S_N2 microwave reaction was performed in an aqueous environment to yield a new heterocyclic compound, which was then converted to the novel luminol derivative via microwave irradiation in the presence of hydrazine (see below).

Preliminary characterization shows promise for a new, cost-effective luminol product that is highly chemiluminescent. It is also shown that this synthesis requires no chromatographic techniques or tedious workup. Finally, the product shows promise for polymerization, which would lead to higher sensitivity among measurements and lower detection limits in various biological assays.

PERFORMANCE UNDER A DELAY-DISCOUNTING TASK PREDICTS THE ESCALATION OF COCAINE SELF-ADMINISTRATION IN FEMALE RATS

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Clinical evidence suggests that impulsivity may influence treatment outcomes in drug addicts. In addition, previous preclinical studies indicate that impulsivity can predict the acquisition, extinction, and reinstatement of cocaine seeking in rats.

The purpose of the present study was to extend these results and examine the effects of impulsivity during the escalation of cocaine self-administration in rats. Female rats were initially screened for impulsivity using a delay-discounting procedure. Subsequently, rats were catheterized and trained to lever-press for cocaine (0.4 mg/kg, i.v.) under a fixed-ratio 1 (FR1) schedule during short-access (ShA) 2-hour sessions until they reached the acquisition criteria. Rats were then allowed access to one of three randomly selected doses of cocaine (0.2, 0.8, or 3.2 mg/kg) under a progressive-ratio (PR) schedule of reinforcement. Subsequently, they were returned to FR1

schedules (0.4 mg/kg), and access was increased to 6 hours (LgA) for 21 days. Following extended access, ShA FR1 and PR dose conditions were reinstated.

Our results indicate that HiI rats increased their cocaine intake during escalation when compared with their LoI counterparts. Furthermore, HiI rats increased ShA intake following LgA while LoI did not. During pre- and post-escalation under the PR conditions, HiI rats increased their number of infusions while the number of infusions for LoI rats did not change. These results lend support to the notion that impulsivity influences excessive drug taking.

SEXUAL DIMORPHISM IN THE AMBUSH BUG

(*Phymata americana*)

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The ambush bug *Phymata americana* is a cryptic, predatory insect that has an unusual pattern of mating behavior. Males engage in a prolonged period of post-copulatory mate guarding. Compared with most insects, male ambush bugs make a large investment in each mating opportunity, which suggests that males may be relatively selective when choosing mates.

Paired and unpaired ambush bugs were collected from goldenrod near Collegeville, MN and Waseca, MN in late August. We measured the thorax width, abdomen length, abdomen width, and dry mass. The right foreleg, middle leg, and hind leg of each insect were mounted on a slide and lengths of the femur, tibia, and tarsus were determined. Paired and unpaired males and females were compared within populations for evidence of assortative mating and mate choice. Males and females were compared to quantify the degree of sexual dimorphism. Ambush bugs from Collegeville and Waseca were compared to determine whether geographical variation was present. Sexual dimorphism was evident. Females were larger than males for all morphometrics and females were almost twice as heavy as males. Relative to a constant metric, thorax width, the legs of males were longer than the legs of females.

There was no correlation between the size of males and females in mating pairs, indicating an absence of assortative mating. Mate choice based on size was not evident because paired and unpaired males were not significantly different for any of the parameters, which was also true of paired and unpaired females.

CHARACTERIZATION OF PERIPHERAL NEUROVASCULAR CAPILLARY STRUCTURE

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Neurovasculature is critical to the peripheral nerve survival. Neurovascular development is well characterized, but little is known about the architecture of the microvasculature and capillary beds of peripheral nerves.

Peripheral nerves from the lower extremities were attained from Wistar Kyoto rats and fixed by glutaraldehyde infusion into the vasculature. The vasculature was subsequently filled with PU4ii polyurethane-based resin to create a three-dimensional cast of the vasculature. The nerves were dissected once the resin was completely polymerized. The nerves were placed in concentrated potassium hydroxide and progressively monitored via light microscopy. The nerves were stained with osmium, critical-point dried, sputter coated with gold palladium, and analyzed via scanning electron microscopy. Partially digested and completely digested specimens were analyzed. The methodology is relatively novel for examining high-resolution lower extremity neurovasculature of the mammal model and may provide information related to regenerative medicine and reconstructive surgery.

DISCOVERING THE STRUCTURAL INTERACTIONS BETWEEN POTENTIAL ANTI-CANCER AGENT NSC 652287 METABOLITE AND 2'-DEOXYGUANOSINE

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The focus of this research was to characterize the cross-links that form between 2'-deoxyguanosine of DNA exposed to 5-[5-(5-formyl-2-thienyl)-2-furyl]-2-thiophenecarbaldehyde (NSC 629035), the proposed active metabolite 2,5-bis (5-hydroxymethyl-2-thienyl) furan (NSC 652287). NSC 652287 is derived from a family of thiophenes that are found in plants and have been used in medicine for therapeutic uses such as antibiotics, antifungal compounds, and antiviral compounds.

Many of the details about how NSC 652287 reacts with DNA are unknown. NSC 629035 was examined for its activity toward 2'-deoxyguanosine. Reactions with calf-thymus DNA and sequence-specific oligonucleotides were also explored. The methods used in this research were derived from previous work done on similar alkylating compounds including crotonaldehyde and diepoxybutane. Samples were analyzed for their reactivity by chromatography methods including LC/MS. Analysis of LC/MS data has shown that NSC 652287 reacts with the calf-thymus DNA.

**EXTENT OF *PHRAGMITES AUSTRALIS*
HAPLOTYPE M (EURASIAN) CRYPTIC
INVASION IN THE MINNESOTA RIVER VALLEY**

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Phragmites australis (Cav.) Trin. ex Steud. is an aquatic grass species associated with wetlands throughout much of North America. The cryptic invasion of these environments by the introduced Eurasian *Phragmites australis* haplotype M over the past two centuries has been reported recently. In addition, the aggressive expansion of clonal *Phragmites australis* populations in many Minnesota wetlands has been noted in recent decades. Wetland scientists have often speculated that these invasive populations may represent non-native strains that are morphologically indistinguishable from native type, now recognized as *Phragmites australis* subsp. *americanus*.

In this study, we investigated the extent of Eurasian *Phragmites australis* haplotype M establishment in the Minnesota River valley. Populations were identified and sampled along transects placed at intervals of 10 to 20 miles along the river corridor from Big Stone Lake (Ortonville, MN) to the confluence of the Minnesota and Mississippi Rivers (St. Paul, MN). Haplotype and native/non-native status was determined for each specimen by PCR-RFLP analysis of two cpDNA regions.

**PERFORMANCE COMPARISON OF TWO
PROCESSING TECHNIQUES FOR NEW AND
USED MULTIPLATE HESTER-DENDY
MACROINVERTEBRATE SAMPLERS**

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Different species of aquatic benthic macroinvertebrates have different pollution tolerances. Evaluating these tolerances, by way of species richness and abundance observations, can be an extremely valuable tool to evaluate the health of a stream; however, consistent and successful collection of macroinvertebrates is critical to securing reliable data. Artificial substrates can be used to sample the benthic macroinvertebrates in rivers and lakes.

Our objective was to determine if there is a difference in macroinvertebrate colonization of new (not previously deployed) and old (previously deployed and processed) multiplate hester-dendy samplers (MHDS). Also of interest were two different techniques utilized by students for removing organisms from the new and the previously used MHDS. Some students have historically scraped organisms from the MHDS, while protocol often

calls for the organisms to be picked. We assessed the total number of organisms, total number of families, total number of ephemeroptera, plecoptera, and trichoptera (EPT), and proportion of families considered to be intolerant of perturbation on new and old MHDS, with half of each processed via scraping and the other half via picking. If the re-use of MHDS or the method in which they are sampled affects the quality of data, then the ability to appropriately evaluate the health of a stream becomes impaired. Based on preliminary data, the total number of organisms did not differ between the new and previously deployed MHDS; however, the percentages of EPT are significantly different between the new and the previously deployed MHDS.

**PARTIAL AMELIORATION OF THE PHENOTYPE
ASSOCIATED WITH DMD THROUGH AN
INCREASE IN VASCULATURE**

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Duchene Muscular Dystrophy (DMD) is an X-linked recessive disease in which the gene that codes for the protein dystrophin is missing. Dystrophin is a membrane-stabilizing protein and absence of it leads to the damage of the muscle membrane, resulting in degradation of the muscle fiber and replacement with fibrous connective tissue. A functional role of dystrophin has also been found in the smooth muscle of the vasculature and its deficiency leads to vascular deficiency and abnormal blood flow. The decreased circulation may induce a state of functional ischemia, increasing the pathogenesis.

For an effective form of therapy of DMD, both the muscle and the vasculature need to be repaired. For this reason, *mdx* (DMD model mice) with increased vasculature were created by heterozygous knocking out *Flt-1*; an inhibitory receptor for angiogenesis. As expected, the *mdx* mice with increased angiogenesis (*mdx:Flt-1^{+/-}*) displayed a better phenotype compared with the *mdx* mice. The *mdx:Flt-1^{+/-}* were shown to have decreased fibrosis, calcification, membrane permeability, and centrally located nuclei. They were also shown to have an increase in whole body force production. Consequently, there was also an increase in type II fibers. These data suggest that increasing the vasculature in DMD may be able to partially ameliorate the phenotype associated with the disease.

CHARACTERIZATION OF CHOROID PLEXUS IN ZEBRAFISH (*Danio rerio*)

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The choroid plexus (CP) is an organ in the ventricles of the brain that produces and circulates cerebral spinal fluid. The CP maintains the blood-cerebral spinal fluid (blood-brain) barrier with respect to ions, growth factors, neuromodulatory hormones, and drugs. CP abnormalities are associated with Alzheimer's disease, schizophrenia, and aging (Emerich et al, 2006). Here, we characterize the choroid plexus stem cell niche in zebrafish. Using a new, tissue specific enhancer trap transgenic line of zebrafish, MnET16, we show that cells expressing green fluorescent protein (GFP) are choroid plexus epithelial cells. In the mouse the choroid plexus stem cell niche is located in the dorsal lip of the hindbrain and is molecularly defined by the protein Gdf-7 (Landsberg et al, 2005. Hunter and Dymecki, 2007). Therefore, using *in situ* hybridization techniques, we characterized the expression of zebrafish Gdf-7 as a presumptive choroid plexus stem cell niche marker in this animal. Gdf-7 was used as a reference for the dynamic and real-time imaging of GFP-tagged CP expression in the MnET16 transgenic line. Expression patterns of Gdf-7 appear to resemble expression of the gene in chick and mouse (Alexandre et al 2007, Hunter and Dymecki 2007). In conclusion, this data suggests that MnET16 is a new model for future choroid plexus research.

EFFECTS OF COCAINE HYDROLASE ON THE ESCALATION OF COCAINE-SEEKING BEHAVIOR IN RATS.

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Cocaine hydrolase (Albu-CocH), an engineered efficient mutant form of human butyrylcholinesterase, blocks cocaine-induced reinstatement of drug-seeking behavior in rats.

In this study, the effects of Albu-CocH on the escalation of cocaine self-administration were investigated. Initially, rats learned to lever-press for a food reward. Subsequently, the rats were trained to self-administer cocaine during daily 2-hour sessions under a fixed-ratio 1 schedule of reinforcement (0.4 mg/kg, i.v.). After 3 days of stable responding (30 infusions/session), the session length was extended to 6 hours for 21 days. For the first 7 days of the 6-hour sessions, Albu-CocH was administered i.v. 30 minutes prior to the start of the session. This was followed by 14 days when no enzyme treatment was administered, and these results were compared with a group that received no injections. Responding of the enzyme-treated rats during the first 7 days of escalation was elevated over the responding of the controls. With suspension of the enzyme treatment during the final 14 days of the escalation period, there was a difference in responding between the groups.

The marked elevation of responding after the first few days of enzyme treatment suggests that the rats were attempting to circumvent the effects of the enzyme. To explore this hypothesis, higher doses of Albu-CocH are being tested to determine whether they will reduce escalation of cocaine self-administration.

NOTES