SCIENCE COLLABORATIVE RESEARCH PROGRAM

S C R P

IX SYMPOSIUM

SCHEDULE AND ABSTRACTS OF STUDENT-FACULTY RESEARCH

09/24/04

SYMPOSIUM ON COLLABORATIVE SCIENCE RESEARCH

SEPTEMBER 24, 2004
1:00-5:10PM
CURREY LECTURE HALL, SPARKS CENTER

TIME	STUDENTS
1:00-1:30pm	Poster Viewing (foyer of Sparks)
1:30	Andrea Countner
1:45	Kara Michels
2:00	Amanda Rice
2:15	Kevin Dean
2:30	William Sandbo
2:45	Joseph Lambert
3:00-3:30pm	Poster Viewing (foyer of Sparks)
3:30	Bobbi Wright
3:45	Alexis LaChapelle and Alex Compton
4:05	Jason Oost
4:20	Johnna Jager
4:35	Travis Harris
4:50	Samantha Lantz and Joel Shinn
5:10pm	Adjourn

Students from the 2004 Science Collaborative Research Program (SCRP) will present their work at our 9th annual in-house symposium on undergraduate research. Students giving single presentations have 12 minutes to deliver their prepared remarks with 2-3 minutes for questions. Those student are paired have 16 minutes for their joint presentations followed by 3-4 minutes for questions. You are all welcome to join us for all presentations or a portion of them. This is a particularly good opportunity for students interested in the research program to discover the excitement of original research.

RESEARCH TITLES AND AUTHORSHIP

THE EFFECT OF CYTOCHROME P-450 MONOOXYGENASE INHIBITORS ON STOMATAL OPENING INDUCED BY REDUCED CO_2 CONCENTRATIONS UNDER RED LIGHT IN *Vicia faba* LEAF EPIDERMIS
Andrea Countner and Gary Tallman

ANALYSIS OF AUXIN SENSITIVITY OF THERMOTOLERANT GUARD CELL PROTOPLASTS USING PLASMID REPORTER CONSTRUCTS
Kara A. Michels and Gary Tallman

COBALT MEDIATED CYCLOANNULATION OF 3,3 & 6,6 PARA BIS-DIYNES
Amanda Rice and Jeffrey Willemsen

LASER LIGHT SCATTERING INVESTIGATIONS OF THE ISOBUTYRIC ACID/ WATER BINARY LIQUID MIXTURE
Kevin M. Dean and J. Charles Williamson

HOLOGRAPHIC INTERFEROMETRY WITH A DIGITAL PHOTO CAMERA
William J. Sandbo and Mark Beilby

TEMPERATURE DEPENDENCE ON THE INTERNAL FRICTION OF METALLIC GLASS FOR POSSSIBLE USE IN LIGO
Joseph Lambert and Mark Beilby

KINETIC CHANGES RESULTING FROM POLE USE IN LOADED DOWNHILL WALKING
Bobbi Wright and Julianne Abendroth-Smith

Xenopus laevis OOCYTE MATURATION, INDUCED BY PROTEIN KINASE A INHIBITOR (PKI), IS BLOCKED BY THE HERBICIDE 2,4-DICHLOROPHENOXY ACETIC ACID
Alexis LaChapelle, Alex Compton, and Barbara Stebbins-Boaz

THE HERBICIDE, 2,4-DICHLOROPHENOXYACETIC ACID, BLOCKS THE ACCUMULATION OF THE ONCO-PROTEIN, C-MOS KINASE, IN *Xenopus laevis* OOCYTES
Jason J. Oost and Barbara Stebbins-Boaz

CARDIOVASCULAR DRIFT: BIOENERGETICS AND METHODOLOGICAL CONSIDERATIONS
Johnna Jager, Kelsie Hendrickson, and Stasinos Stavrianeas

INHIBITION OF CHYMOTRYPSIN BY MERCURY(II) DEPENDS ON BUFFER, pH, AND HISTIDINE SIDE CHAINS
Travis Harris and Todd Silverstein

THE BEHAVIORAL ECOLOGY OF CASPIAN TERNS (*Sterna caspia*): DIET AND MOVEMENT

Samantha Lantz, Joel Shinn, and David Craig

THE EFFECT OF CYTOCHROME P-450 MONOOXYGENASE INHIBITORS ON STOMATAL OPENING INDUCED BY REDUCED CO_2 CONCENTRATIONS UNDER RED LIGHT IN *Vicia faba* LEAF EPIDERMIS

Andrea Countner and Gary Tallman

Guard cells, found in plants, control the dimensions of the openings of leaf stomata, and thereby, rates of transpiration and photosynthesis. Lowering concentrations of CO_2 around stomata illuminated with red light activates stomatal opening, however, the mechanism is unknown. One hypothesis is that reduced CO_2 concentrations trigger activation of cytochrome P-450 mono-oxygenases (CytP-450) that catalyze the metabolism of endogenous guard cell abscisic acid (ABA), a hormone that prevents stomatal opening. Stomata in epidermis detached from leaves of *Vicia faba* L. were illuminated with red light or maintained in darkness and exposed to CO_2-free air in solutions containing or lacking the CytP-450 inhibitors: ancymidol or paclobutrazol. Stomatal opening was monitored microscopically over a four hour period. At different concentrations, ancymidol (1mM) and paclobutrazol (5μM) fully inhibited stomatal opening normally induced by light and reduced CO_2 concentrations. The results are consistent with the hypothesis that lowering CO_2 around stomata illuminated with red light activates stomatal opening by triggering catabolism of endogenous guard cell ABA.

ANALYSIS OF AUXIN SENSITIVITY OF THERMOTOLERANT GUARD CELL PROTOPLASTS USING PLASMID REPORTER CONSTRUCTS

Kara A. Michels and Gary Tallman

A phenomenon referred to as thermotolerance has been observed in some species of plants. Thermotolerance is characterized by halted plant growth in high temperature conditions. It is suspected that insensitivity to hormones such as auxin and cytokinin is responsible for the onset of thermotolerance, as these hormones normally induce growth responses. Protoplasts from the guard cells of *Nicotiana glauca* are easy to isolate and efficient for cellular studies on thermotolerance because their response has been previously studied and recorded. This research built upon these foundations and examined cells in two temperature environments: 32°C and 38°C, the latter a laboratory simulation of a thermotolerance inducing environment. Previously, protoplasts incubated at 32°C dedifferentiated and divided when treated with auxin and cytokinin; those cells treated similarly at 38°C did not fully reenter the cell cycle but survived longer than their 32°C counterparts. For this research it was hypothesized that the cells incubated at 38°C were insensitive to auxin. Auxin sensitivity was assayed using plasmid reporter constructs with an auxin-induced promoter. The reporter in these studies was green fluorescent protein (GFP). Once transformed with this construct (GH3-sGFP), cells that were sensitive to auxin (this was expected at 32°C but not at 38°C) were expected exhibit fluorescence when observed with fluorescence microscopy, seeing that the reporter gene should be transcribed and translated. This research revolved greatly around perfecting transformation techniques to maximize cell survival. As soon as this procedure was satisfactory the percent of protoplasts fluorescing was recorded for protoplasts with and without an auxin treatment and for both 32°C and 38°C. Most of the data support the hypothesis that thermotolerant protoplasts are not auxin sensitive as the percentages of cells fluorescing at 38°C were very low or zero. However, while fluorescence was noted at 32°C, the expected onset of division at this temperature was not seen in cells that were treated for transformation. In the immediate future this research would likely assay the effects of cytokinin-induced and constituent promoters, new transformation methods, and determine optimal GFP response times.

Cobalt Mediated 2+2 Annulation on 3,3 and 6,6 (*para*) Bis-Diynes

Amanda Rice and Jeffrey John Willemsen

A successful two-step synthesis' of TMS protected 3,3 and 6,6 para acylic bis-diynes completed the preparation for a 2+2 cycloannualation reaction. Both reactions will be analyzed after the cobalt mediated annulation using dicarbonylcyclopentadienylcobalt via NMR spectroscopy. A variety of *para, ortho and meta* bis-diynes have undergone 2+2 annulation for comparative analysis. This includes the regiochemical characterization and structural elucidation of cyclophanes via x-ray crystallography.

LASER LIGHT SCATTERING INVESTIGATIONS OF THE ISOBUTYRIC ACID/ WATER BINARY LIQUID MIXTURE

Kevin M. Dean and J. Charles Williamson

Previously, researchers have neglected the different types of light scattering involved in two phase liquid/liquid solutions. Consequently, these assumptions have resulted in accurate data for the determination of the phase change temperature, especially near the critical point. With a better understanding of Rayleigh and Mie scattering, and an alternative approach to account for these light interactions, one can more accurately determine the phase diagram involving liquid/liquid solutions. We used 4 light detectors to account for the different types of light scattering, set up respectively at incident, 30 degrees, 90 degrees, and 135 degrees. We evacuated all of the atmospheric gases from the sample by a series of freeze thaws, and flame sealed each ampule. We were able to take .01 degree Celsius steps, and allowed each sample to equilibrate at each respective temperature for 8 minutes. Further research is planned in an effort to better understand the microscopic interactions involved in liquid/liquid phase changes.

DIGITAL HOLOGRAPHY USING A DIGITAL PHOTO CAMERA

William J. Sandbo and.Mark Beilby

Holographic interferometry (HI) utilizes the constructive and destructive interference properties of light to view the distortion of an object that is under stress. The resulting pattern of light and dark fringes show where the object has moved on the order of a half a wavelength of light. Thus, HI is appropriate for investigating movements that the naked eye or traditional ways of measurement cannot detect. There are, however, a number of ways and techniques of producing an interference pattern. A novel system using the technique of speckle pattern digital holography was constructed using a consumer digital camera and a diode laser as the light source. The system was tested using a flat, clamped circular metal sheet as the object and successful interference patterns for both static stressing and vibration were obtained. The flexibility of this system will allow for the future study of vibrations of many different objects.

TEMPERATURE DEPENDENCE ON THE INTERNAL FRICTION OF METALLIC GLASS FOR POSSIBLE USE IN LIGO

Joseph Lambert and Mark Beilby

Research has been conducted to determine vibrational properties of a new material called metallic glass. The Q factor is one such property that tells how well a material conserves vibrational energy. A high Q factor indicates little transfer of vibrational energy to heat due to internal friction. It has been found that metallic glass has a relatively high Q factor of 13000±3000 at room temperature. It is believed that the high Q is due to the amorphous structure of metallic glass. The goal of this research was to test this belief by heating strips of metallic glass above the crystallization point and re-determining the Q factor. The metallic glass was heated for one hour at approximately 700°C and measured a Q value of 14000±4000. A similar sample was heated for one hour at approximately 400°C and measured a Q value of 11000±2000. Preliminary findings suggest that the Q value does not change within the errors of the experiment when metallic glass is heated past the crystallization point. Though there does not seem to be a significant change in the Q value when heated, the resonant frequencies of the metallic glass increased significantly by a factor of approximately 1.5 and the time it took to damp down decreased significantly. This shows that the stiffness of the metallic glass increased when heated. More tests need to be done to be certain that the metallic glass was in fact crystallized.

KINETIC CHANGES RESULTING FROM POLE USE IN LOADED DOWNHILL WALKING

Bobbi Wright and Julianne Abendroth-Smith

Hiking is a recreational past time that often takes the individual away from civilization for multiple days. It is then important that they carry supplies; therefore, many hikers carry a loaded backpack. To alleviate some of the stress placed on the joints, walking poles have emerged as popular aides to use while backpacking. In this study, the effectiveness of walking poles is examined in downhill hiking while carrying a loaded pack. METHODS: Participants walked a 24 meter ramp with a downhill grade of 20 degrees (36%) while wearing backpacks loaded with 25% of their body weight. A force plate was located on the downhill portion of the ramp. The participants walked the ramp for eleven trials with poles and again without poles. Ground reaction and breaking forces were collected for each trial. Electromyography (EMG) was used to measure muscle activity for each trial, with surface electrodes placed on the vastus lateralis, rectus femoris, and biceps femoris of the participants. RESULTS: The results demonstrated a statistically significant decrease in the vertical force with pole use. Breaking force was less on average with pole use, but was not statistically significant. The activity of the biceps femoris was significantly reduced with pole use. The change of the rectus femoris was not significant, but the activity increased on average with pole use. CONCLUSION: The results demonstrate that pole use reduces force on the joints in loaded downhill walking. However, an increase in muscle activity at the rectus femoris may result in increased muscle soreness.

XENOPUS LAEVIS OOCYTE MATURATION, INDUCED BY PROTEIN KINASE A INHIBITOR (PKI), IS BLOCKED BY THE HERBICIDE 2,4-DICHLOROPHENOXY ACETIC ACID

Alex Compton , Alexis LaChapelle, and Barbara Stebbins-Boaz

The development of animal embryos is preceded by the healthy maturation of haploid germ cells. In female *Xenopus laevis*, oocytes undergo maturation via a signal transduction pathway triggered by progesterone, which leads to the progression through meiosis and the formation of a fertilizable "egg." The commonly-used herbicide, 2,4-Dichlorophenoxyacetic acid (2,4-D), has been shown to inhibit oocyte maturation by interfering with the progesterone pathway. This study set out to more accurately define the substrates through which 2,4-D acts. The heat-stable protein kinase A inhibitor (PKI), a protein that blocks the activity of the G2/M-arresting protein kinase A (PKA), was used to progress the oocyte through M phase without the use of progesterone. By injecting oocytes with PKI and then incubating in 2,4-D, we hypothesized that the substrate(s) targeted by the herbicide could be limited to points upstream or downstream of the PKA-PKI binding site. Western analysis was used to assess the activity levels of certain key proteins of the maturation pathway. mos, MAPK, and cdc2 proteins were all detected using autoradiography in PKI-injected oocytes treated with 2,4-D. The results showed inactivated cdc2 and decreased levels of mos activity, indicating that 2,4-D blocked PKI-induced oocyte maturation. Findings from the MAPK detection were disputable due to conspicuous control outcomes. The ability for 2,4-D to continue blocking maturation after PKI injection suggests that the point at which the herbicide acts in the pathway may be downstream of PKA (or at PKA itself). However, it is still possible that 2,4-D acts at multiple points both downstream and upstream of PKA. These data may help uncover the process through which 2,4-D harms successful oocyte maturation.

THE HERBICIDE 2,4-DICHLORPHENOXYACETIC ACID BLOCKS THE ACCUMULATION OF THE ONCO-PROTEIN, CMOS KINASE, IN *XENOPUS LAEVIS* OOCYTES

Jason Oost and Barbara Stebbins-Boaz

The presence of the herbicide, 2,4-Dichlorophenoxyacetic acid (2,4-D), has previously been shown to manipulate, and eventually halt, the maturation pathway normally induced by progesterone in *Xenopus laevis* oocytes. In this pathway, the onco-protein, cMos kinase, is normally translated and accumulates in the oocyte 3 to 6 hours after it is induced by progesterone. This study investigated the effects of 2,4-D on the translation and accumulation of cMos. To determine if cMos protein itself was being degraded, oocytes were pre-incubated in progesterone until maturity, then incubated at several different time increments in either theknown translational inhibitor cyclohexamide, or 2,4-D. The presence of cMos was determined through Western blot analysis, which showed no significant degradation of the protein after translation. Again using Western blot analysis, the dephosphorylation of cdc2, which is indirectly caused by the accumulation of cMos, was affirmed indicating that cMos was functional. To determine whether cMos accumulation would be affected by 2,4-D when added to oocytes earlier in their progesterone incubation period, a second experiment was carried out. Oocytes were again incubated in progesterone with the addition of 2,4-D at several time increments. Using the same methods as the previous experiment, it was determined that 2,4-D added to oocytes early in the incubation did not accumulate significant cMos, and only contained inactive, P-cdc2. However, oocytes that had 2,4-D added later in their incubation period accumulated cMos and cdc2 at high levels similar to control oocytes in only progesterone. These results indicate that 2,4-D does not degrade the cMos protein once it has accumulated. However, the results do show that at some point before cMos accumulation, likely at translation, 2,4-D is halting expression of the protein.

CARDIOVASCULAR DRIFT: BIOENERGETICS AND METHODOOGICAL CONSIDERATIONS

Johnna Jager, Kelsie Hendrickson, and Stasinos Stavrianeas

Cardiovascular drift (CVD) is a commonly observed response to prolonged exercise at moderately high intensities (>60% VO_{2max}). Although many studies have explored the possible causes of CVD, no reports of the metabolic cost of CVD were found. The project aims at examining potential changes in energy consumption upon onset of CVD at intensity just below lactate threshold (LT). Phase 1 of the project involves the establishment and validation of exercise and testing protocols. We created calibration standards for the measuring of mechanical work on a cycle ergometer. We generated standard curves for the measurement of blood glucose and blood lactate levels during exercise, and compared our results using two methods (spectrophotometer vs. strips). Finally, we validated a protocol that allowed us to establish LT and incorporate all the necessary physiological measurements. Standard curves for blood lactate and blood glucose measured spectrophotometrically were very accurate ($r=0.991$ to $r=0.9999$). Blood glucose measurements were almost identical between the spectrophotometer and the Accu-Chek™ strips ($r=0.97$), whereas the Prestige™ system yielded significantly less accurate results ($p<0.05$ when compared to the two methods above). Similarly, the lactate values were very similar between the spectrophotometer and the Accusport™ lactate analyzer ($r=$ _____). We finalized most of the methodology to be used in this study, and are confident that if such changes are detectable we will be able to identify and quantify them. The completion of the lactate and glucose experiments and the inclusion of the newly acquired metabolic gas analyzer will allow the transition to the data collection phase of this project.

0.075

INHIBITION OF CHYMOTRYPSIN BY MERCURY (II) DEPENDS ON BUFFER, PH, AND HISTIDINE SIDE CHAINS

Travis Harris and Todd Silverstein

To study the toxic effects of mercury, $HgCl_2$ was incubated with the digestive enzyme, chymotrypsin, under a variety of conditions. $HgCl_2$ causes denaturation of chymotrypsin, and also inhibits the enzyme left in solution. A titration curve, prepared by spectrophotometrically measuring the rate of precipitation at 350 nm, suggests that a group with pKa 7.6 is responsible for mercury-induced chymotrypsin aggregation. It was found that the aggregation is buffer dependent: HEPES buffer yielded steady increases in precipitation as pH increased from 6.7 to 8.3, while there was no reaction with Tris buffer at pH<9, or with glycine at any pH from 9-10. Tris buffer did support mercury-induced chymotrypsin precipitation at pH 9, but the rate was much slower than with TAPS and CHES buffers at the same pH. The percentage of chymotrypsin left in solution after precipitation and filtration decreased as $[HgCl_2]$ increased up to 1.5 mM, after which it remained at about 10%. The enzymatic activity of the chymotrypsin remaining in solution was tested by measuring pNPA hydrolysis ($\Delta A_{400}/\Delta t$). Chymotrypsin inhibition increased with $[HgCl_2]$, up to 1 mM, after which the rate of hydrolysis remained the same. Michaelis-Menten plots showed that k_{cat} is about 0.2 +/- 0.1 min^{-1} independent of Hg^{2+}, whereas K_m increased from 5 mM (control) to 20 mM (at 2 mM $HgCl_2$), suggesting that Hg^{2+} behaves like a competitive inhibitor of chymotrypsin. Tests with the histidine-blocking reagent DEPC demonstrated that greater than 5 mM DEPC completely protects chymotrypsin from mercury-induced precipitation. However, 1 mM DEPC actually enhanced precipitation, indicating that partial histidine blockage may direct Hg^{2+} to more critical histidine residues.

THE BEHAVIORAL ECOLOGY OF CASPIAN TERNS (*Sterna caspia*): DIET AND MOVEMENT

Samantha Lantz, Joel Shinn, and David Craig

We investigated the behavioral ecology of Caspian terns (*Sterna caspia*) with a specific interest in improving techniques associated with diet and movement. Diet studies consisted of two components: an analysis of observer accuracy in the length estimates of bill loads, and the construction of an identification guide to the forage fish of piscivorous seabirds on the Pacific coast. Data on observer accuracy showed significant variance between observers, indicating a need for a standard training protocol for use in field studies. Our fish forage guide describes how to accurately identify more than 40 species of fish commonly held in the bills of 15 species of Pacific seabirds (alcids & sternids). Movement and migration research was focused on piloting a safe design of a harness system for attaching satellite telemetry tags to terns. Satellite tags were attached to five terns at Potholes Reservoir, WA, on May 13, 2004 using yoke-shaped neoprene harnesses. Three of the five terns died within a month of tagging due to harness complications, prompting a more thorough investigation of harness design. A modified yoke design was applied to a Caspian tern held in captivity for close observation. At four weeks minor complications arose, so a third design is currently being tested. With ongoing modifications the harness design should be safe for at least one year. Our exploration of both diet and movement techniques is advancing knowledge of the behavioral ecology of Caspian terns. A better understanding of Caspian tern diet and movement is essential to the continued conservation of this species.