

1998

Winchell Posters

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Recommended Citation

(1998). Winchell Posters. *Journal of the Minnesota Academy of Science*, Vol. 62 No.2, 24-30.
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ANTIVIRAL EFFECT OF BETULIN AGAINST HERPES SIMPLEX VIRUS(HSV-1 AND HSV-2)

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Betulin, a pentavalent triterpenoid compound extracted from Minnesota White Birch Bark, has been found to have significant antiviral activity against Herpes Simplex Viruses in human neuroblastoma (SK-N-MC) cells. Preliminary results indicate that Betulin is 50-100 times more effective as compared to acycloguanosine (Acyclovir®) at inhibiting replication of HSV-1 and HSV-2. Our results indicate that Betulin, at its highest concentration, is non-toxic and non-growth-inhibitory to SK-N-MC cells. Cytotoxicity was determined by growing SK-N-MC cells in supplemented MEM-Betulin solution. Our studies indicate that Betulin post-treated cells have shown 3 times more protection compared to Betulin pretreated cells, indicating that continued presence of Betulin is required for the inhibition of the virus. Further work is in progress to elucidate the effect of Betulin at the molecular level by increasing the permeability of cell membrane.

SEPARATION AND USEFULNESS OF XYLENOL-ORANGE IN METAL ION TITRATIONS

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The commercially available indicator, Xylenol-Orange, is an impure mixture of starting materials and intermediate compounds along with Xylenol-Orange. The impurities have varying binding properties and yield incorrect results when used in metal ion titrations. Pure Xylenol-Orange is a useful indicator in spectrophotometric titrations that deal with small metal ion concentrations. This study covers the purification, as well as Xylenol-Orange's usefulness in metal ion determination.

COMMUNICATION BETWEEN INCUBATING FEMALES AND THEIR MATES IN THE HOUSE WREN *TROGLODYTES AEDON*

Kristina Blake-Hodek, Anne Cooper, Tom Guy, Henry Kermott, St. Olaf College, Northfield, Minnesota 55057

Male House Wrens periodically approach the nest box and sing during the incubation stage. The female, who incubates alone, appears to wait to exit until the male sings just outside the entrance. The function of bird song, and how it is used for communication between the sexes, is a major interest in Ornithology. The reproductive success of upper vertebrates often depends on communication between males and females. This study tested for a nonrandom association of the female exiting the nest box with male arrival and singing the "nest relief" song. This study was done on private land in southeastern Rice County, MN. House Wrens were observed for 1 h during the incubation stage by using a spotting scope at a distance of 20-30m from the nest box and were not banded. The time of each male song and his distance from the box were noted, along with the female entry and exit times, and how long she was absent from the nest. The results strongly suggest that females do wait for their mate to sing the "nest relief" song before exiting the nest. This communication may benefit the House Wrens since the male can indicate to the female that the area is clear of predators, that he will be present to guard the nest from conspecifics, and can act as a sentinel while the female is foraging.

24 HR. SPARFLOXACIN EXPOSURE RESULTS IN STABLE FLUOROQUINOLONE CLASS RESISTANCE IN BACTEROIDES FRAGILIS

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Sparfloxacin, one of several new fluoroquinolones, shows to be effective against both aerobic and anaerobic bacteria. The potential for resistance after a twenty-four hour exposure to sparfloxacin in *B.fragilis* (ACCT 23745 and ACCT 25285) was determined using an anaerobic in vitro model, 24 hour time kill curves and minimal inhibitory concentration tests. MIC's on both pre- and post-sparfloxacin-exposure isolates were tested for sparfloxacin, trovofloxacin, clinafloxacin, ciprofloxacin, and levofloxacin using NCCLS standard methods. Results show increased MIC's for all drugs tested. Isolates were passed for 10 days and MIC's tested. Results show that the sparfloxacin exposed isolates demonstrate an induced stable fluoroquinolone class resistance with in a twenty-four hour exposure. Further investigation is needed to determine the mechanism of resistance, the susceptibility to other drug classes after sparfloxacin exposure, the potential of resistance in other anaerobic and aerobic organisms, and the potential clinical significance of these findings. (This data was presented at ICAAC in Toronto, September 1997, by Marnie Peterson.)

FACTORS CORRELATED WITH SATISFACTION OF INTERNATIONAL STUDENTS WHILE ATTENDING ST. CLOUD STATE UNIVERSITY

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This study aims to determine those factors strongly correlated with a satisfactory experience as an international student in the United States. A questionnaire was mailed to all persons designated as international students at St. Cloud State University. Level of satisfaction was assessed by evaluating the subjects' responses to a variety of questions concerning their satisfaction in particular areas. The key factors examined in this study are level of social contact in a variety of situations, financial means to accommodate needs, and academic satisfaction. Behavioral information in these specific areas was requested to find correlation between international students' behaviors and perceived satisfaction. It was predicted that orientation of culture of origin (individualist versus collectivist) would be a strong indicator of satisfaction with the experience at St. Cloud State University. It was also predicted that level of social interaction would influence satisfaction, potentially producing an interaction effect with the orientation of culture of origin.

INTERSTITIAL FLUID (ISF) ADENOSINE DURING EXOGENOUS ADENOSINE ADMINISTRATION IN THE ISOLATED RABBIT HEART

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Brief periods of transient myocardial ischemia have been shown to protect the heart during subsequent extended periods of ischemia, a phenomenon known as ischemic preconditioning. In the rabbit heart, the protection afforded by ischemic preconditioning can be mimicked by transient exogenous adenosine administration, presumably as a result of an increase in ISF adenosine. The purpose of the present study was to determine the changes in ISF adenosine during graded intracoronary administration of adenosine in isolated perfused rabbit hearts. Successive adenosine infusions at 3, 6, 10, 20, 50, 100 µM were administered in 5 minute increments while ISF was sampled from the left ventricular myocardium via cardiac

microdialysis. Estimated ISF adenosine increased from 0.07 μM without exogenous adenosine to 0.14, 0.34, 0.55, 1.16, 6.21, 13.50 μM at 3, 6, 10, 20, 50, and 100 μM , respectively. These ISF adenosine levels during graded adenosine infusion compliment a study by Woolfson et al. (*Cardiovascular Res.* 31:148, 1996) which showed a graded decrease in infarct size when hearts were pretreated with similar increasing concentrations of intracoronary adenosine, supporting the hypothesis that an increase in ISF adenosine is an integral component of the reduction in infarct size due to ischemic preconditioning. These data also demonstrate a substantial metabolic barrier for the movement of exogenous adenosine out of the vascular space and into the ISF. Supported by NIH HL-46027.

CONTEXT AND STRUCTURE OF ADVERTISING SONG IN UNMATED MALE HOUSE WRENS (*TROGLODYTES AEDON*)

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We describe the structure of advertising song of unmated males in a population of House Wrens on private land in south-eastern Rice County. Much interest in ornithology is concentrated on the function of bird song, as reproductive success in higher vertebrates frequently depends on proper communication between males and females. Most males in this study were vocal throughout the breeding cycle, but actual song output during the advertising period prior to obtaining a mate appears to be the most intense. Males were provided with nest boxes and were not banded. Recordings were done using directional microphones 10-15 meters away from the singing male for 60 minute intervals. The advertising songs of three males were analyzed using 20 song spectrograms for each male sampled. Analysis was done using Signalize software. Songs were analyzed for consistency, structure, and extent of variation from one song to the next. Behavioral contexts within these variations did occur and all activities and events were noted. The results suggest that individual males repeat a particular song continuously, switch to another, then return to the original. The adaptive significance of this will be discussed at the meeting.

AMPLIFICATION OF cpDNA FROM *MALUS* USING UNIVERSAL PRIMERS

John Crofts and Paul Cabe, St. Olaf College, Northfield, MN 55057

Genetic diversity of important cultivated plants is a crucial issue for breeders and those concerned with germplasm conservation. For fruits like the domestic apple (*Malus domestica*), reliable genetic markers could be used in breeding programs, and for assessing the genetic variability and parentage of cultivars. Highly variable nuclear markers (microsatellite loci) are now available for apples, but attempts to find variable cytoplasmic markers (chloroplast or mitochondrial DNA) have met with limited success. Cytoplasmic markers would be useful in distinguishing maternal vs paternal ancestry, and in quantifying seed dispersal in wild populations.

We used the polymerase chain reaction (PCR) and universal primers available from the literature to amplify non-coding sections of cpDNA from domestic apples. These regions were amplified from DNA isolated from a variety of tissues, including mature leaves and seeds. These regions can now be screened for intraspecific sequence differences.

FIRE HISTORY FOR TWO BIG WOODS LAKES: A PRELIMINARY REPORT

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Causal link(s) between fire, climate, and vegetation remains a topic of considerable research. I approached this question by

reconstructing the local fire history of two lakes, Kelly-Dudley and Hunt, located within what was the Big Woods. I used charcoal concentration and morphology, as well as various sediment physical properties to address this issue. Kelly-Dudley and Hunt Lake varied in the amounts of charcoal deposited but both lakes showed a decline in charcoal approaching the Euro-American settlement horizon. This suggests a gradual change in fire regime consistent with climate driven expansion of Big Woods that occurred 300-400 years ago. Charcoal peaks were present in both lakes after the transition to Big Woods, suggesting that fires may have been present within the Big Woods itself. Kelly-Dudley Lake had much higher L:W ratios than Hunt Lake identifying a difference in fuel type between the two areas studied.

FAR-INFRARED LASER STARK SPECTROSCOPY OF CD₃OD: DETERMINATION OF THE ELECTRIC DIPOLE MOMENT

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Recently, the Fourier transform far infrared (FTFIR) spectra for the totally deuterated isotopic species CD₃OD, were recorded at the Justus Liebig Universität, in Geissen, Germany. Several transitions previously assigned in the laser Stark spectra belonging to the Q-branch multiplet $J_k = J_{11} \leftarrow J_{10} E_2 v_t = 0$ for $J = 13$ and 14 were identified and measured in the FTFIR spectra to an accuracy of ± 6 MHz. By combining this data with the previously assigned work, the μ_a component of the electric dipole moment was calculated for the first time.

The FIR laser Stark spectra were taken using a direct discharge molecular gas laser and a Stark spectrometer. A 10 fit long Pyrex glass tube, separates the anode and cathode chambers. Two copper mirrors are used. With one in the anode chamber having a radius of curvature of 8 m and the other flat mirror located in the cathode chamber. A 45° diagonal brass mirror couples the beam out of the laser cavity and into the Stark spectrometer. The FIR laser Stark spectra, used in this work, were taken with hydrogen cyanide (HCN) as the lasing medium, operating at the $\lambda = 311 \mu\text{m}$ laser line.

The Stark cell consists of a cylindrical stainless steel housing with mylar windows at each end. It encloses two parallel silvered glass plates, separated by 0.051508 ± 0.00005 cm through the use of quartz spacers. An electric field up to 60,000 V/cm can be applied between the Stark plates, allowing molecular absorption lines to be brought into resonance with the laser frequency.

The equations and methods used to calculate the μ_a component of the electric dipole moment for CD₃OD will be discussed, along with the experimental setup used for taking the laser Stark spectra.

CHARACTERIZATION AND COMPARISON OF TWO OAK SAVANNA COMMUNITIES

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Oak savanna communities were widespread in Minnesota before European settlement, but today they have all but vanished. The purpose of this study was to characterize a stand of bur oaks on St. Olaf College property by comparing it with a known oak savanna at Nerstrand Big Woods State Park. Tree density, diameter, age and percent canopy cover were measured at each site. The data suggest that the Nerstrand site is more representative of a typical oak savanna ecosystem while the St. Olaf site is probably an oak woodland. Bur oaks that were cored at St. Olaf were mostly between 80 to 120 years old but there were a few trees old enough to have been present in the mid 1850s, before European settlement.

This suggests the likelihood that an oak savanna ecosystem existed before the time of settlement. If St. Olaf College decides to restore the site to its original condition, it would require a prescribed burning schedule and the opening of the canopy.

THE PHYSIOLOGICAL ROLE THAT THE FK BINDING PROTEIN HAS ON THE RELEASE OF Ca^{++} FROM THE LUMEN OF SKELETAL SARCOPLASMIC RETICULUM

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The sarcoplasmic reticulum (SR) functions in muscle contraction by controlling the Ca^{++} ion concentration bathing contractile muscle filaments. An action potential is sent from an axon terminal on the muscle cell membrane and is propagated to the SR. The receptor site on the SR is the ryanodine receptor, the major focus of our current study. The complete structure and its precise mechanism have not determined at this time. We are currently attempting to determine how certain divalent cations (Mg^{++} and Ca^{++}) and temperature effect the release of Ca^{++} when the FK binding protein is removed from the ryanodine receptor of rabbit skeletal muscle.

AMPLIFICATION OF cpDNA FROM *LEMNA MINOR* USING UNIVERSAL PRIMERS

Kristina Engen and Paul Cabe, St. Olaf College, Northfield, MN 55057

The polymerase chain reaction (PCR) has opened the door to sophisticated studies of population structure and ecology. One drawback is the necessity of DNA primer pairs, which typically have to be developed for each new species studied. Recently, however, researchers have published lists of universal primers which amplify non-coding sections of chloroplast DNA (cpDNA). These non-coding regions offer the potential for variable DNA markers suitable for population level studies, but to date have not been used in many studies.

We have screened a variety of these universal primers for use with *Lemna minor* (duckweed), a common, cosmopolitan aquatic angiosperm. We have optimized PCR to amplify cpDNA fragments of a size consistent with those expected based on work with other taxa. Minimal amounts of plant tissue are required. DNA from single fronds (ca. 1-2 mg fresh tissue) of *L. minor* can be isolated and amplified using this PCR technique. This research opens the door for population level studies using restriction fragment length polymorphisms or direct sequence analysis of the amplified fragments.

ACTIVATION OF THE NOCICEPTIN RECEPTOR IN THE RAT BRAIN

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Nociceptin and its receptor are the most recently discovered members of the endogenous opioid family. Like all other opioid receptors, nociceptin is a G-protein linked receptor. While the receptor has been shown to be present in the rat brain, nociceptin has not yet been shown to activate its receptor. Sections of rat brain mash were incubated with [35 S] GTP γ S and stimulated with nociceptin. The G-protein is unable to hydrolyze the GTP molecule upon further stimulation due to the substitution of sulphur for oxygen in the γ position. GMP/PNP, a GTP analog, was used in the non-specific binding control.

Total binding was appreciably higher than the non-specific and unstimulated binding, thus demonstrating that nociceptin activates its receptor in brain tissue. Preliminary attempts at using the procedure in the production of autoradiographic slides were successful.

We propose that with proper adjustments this assay can be used to demonstrate G-protein activation in a wide variety of G-protein linked processes. The current practice of using tritiated ligand in autoradiography results in exposure times of three to four months. The use of [35 S] GTP γ S reduces the exposure time to about three days, as well as reducing the overall cost of reagents and disposal.

EFFECT OF RETRIEVAL INHIBITION ON CUED AND FREE RECALL MEMORY TESTS

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Participants studied a word list, after which half were instructed to remember the words (R group), and the other half instructed to forget the words (F group). Following study of a second list of words, of which all participants were instructed to remember, memory for words from the second list was tested. Half of the participants from both the R and F groups completed a free recall test for the second list words. The remaining participants completed a cued recall test for the second list words, with four randomly selected words from the first list. On the free recall test, F group participants recalled more second list words than the R group participants. On the cued recalled test, there was no significant difference in recall between groups. The free recall results demonstrate that a "forget" instruction inhibits the retrieval of the first list of words, causing less proactive interference from those words in the recall of second list words. On the cued recall test, a presentation of first list words released the inhibition acting on the remaining first list words for the F group, increasing their proactive interference on the recall of list two words. The added proactive interference for the F group on the cued recall test is similar to the proactive interference experienced by the R group. These results are consistent with the retrieval inhibition view of directed forgetting.

DETERMINING WHETHER MUTANT ALLELE FREQUENCIES IN A CAT POPULATION REMAIN CONSTANT OVER TIME

Marcella Goff, Hamline University, 1536 Hewitt Ave., St. Paul, MN 55104

I surveyed a sample of the Minneapolis-St. Paul cat population and compared the results to two other surveys conducted on the same population during the past 13 years. The purpose was to determine whether the allele frequency of several different loci controlling coat phenotype had changed significantly over this time period. To determine whether human selection had a significant effect on mutant allele frequencies, I also compared the allele frequencies of cats surveyed at veterinary clinics to those at humane societies. In addition, I tested for random breeding at both the orange and piebald loci. Of the seven loci observed, four of the mutant allele frequencies (dominant white, dilute, and blotched tabby) demonstrated significant shifts toward lower frequencies, whereas, the Abyssinian tabby allele exhibited a shift towards a higher frequency. The agouti allele increased significantly and subsequently decreased in frequency. Veterinary clinic and human society cat populations exhibited significant differences for the dominant white and agouti mutant alleles. The results from this study indicate that allele frequencies in stable populations can change significantly over short periods of time.

EFFECTS OF GROWTH HORMONE REGULATION BY SOMATOSTATIN IN DIABETIC SPRAGUE-DAWLEY RATS

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Along with lack of insulin, type I diabetics experience high levels of growth hormone secretion. This has been associated

with metabolic instability, insulin resistance, the dawn phenomenon, diabetic nephropathy, and proliferative retinopathy. Somatostatin and its analogues have been shown to decrease growth hormone levels and increase insulin sensitivity. Since hormones such as insulin and growth hormone affect metabolic rate and body size, these characteristics should be expected to vary in diabetic rats due to decreased insulin and increased growth hormone levels. The purpose of this project was to determine the effects of Somatostatin on basal metabolic rate, body length, and body size in male diabetic rats. Organ weights were also determined to see if Somatostatin affects the heart, liver, spleen, and kidneys. Four groups of animals were used: diabetic + somatostatin, diabetic + placebo, nondiabetic + somatostatin, and nondiabetic + placebo. Somatostatin (50 ug/kg body weight) or saline (0.9%) were injected subcutaneously twice daily for 28 days. Basal metabolic weights were measured using a PhippsBird metabolic chamber, after which the animals were sacrificed and the heart, liver, spleen, and kidneys were removed and weighed. These values were then compared among the four groups.

LOW TEMPERATURE SPUTTERING YIELD OF Ag-Cu ALLOYS FOR 200 eV Ar⁺

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During recent experiments involving 200 eV Ar⁺ ion bombardment of Ag-Cu alloys held at -50°C, some perplexing results have surfaced which have important consequences in the field of "sputtering". Interestingly, the yields (number of ejected atoms per incident ion) of all alloy compositions were equal to the yield of pure Cu. Usually, the yield of these types of materials is very unpredictable, but our experiments show that if the sample is cooled down to a certain temperature, the yield becomes predictable. These results have broad ramifications in the technological application and fundamental understanding of "sputtering" of multi-element multi-phase materials.

HABITAT USE AND SOCIAL ORGANIZATION OF JUVENILE COHO SALMON (ONCORHYNCHUS KISUTCH) DURING OVER-WINTERING PERIOD IN A LAKE SUPERIOR STREAM

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Lake Superior coho salmon have supported a recreational fishery for more than twenty years; however, recent declines in harvest have increased interest in the status of these poorly studied populations. Management of Lake Superior coho focuses on the vulnerable stream life phase and has been greatly influenced by research from the native range (Pacific coast) of this species. Unfortunately, insights from Pacific streams may have limited value in Lake Superior streams as environmental conditions in the two regions differ profoundly. For example, winter floods are common in streams along the Pacific coast, but rare events in Wisconsin. Because floods and high water velocities can influence fish behavior, there are likely to be significant differences in the winter ecology of juvenile coho from Pacific and Lake Superior streams. This study was motivated in part by this possibility. Specific objectives were 1) to describe the distribution and social organization of coho in a Lake Superior tributary and 2) compare these patterns to published information on Pacific coast populations. Field research completed in February and March of this year indicates that the behavior of wintering juvenile coho in Lake Superior is more similar to the behavior seen during the summer in Pacific streams than in the winter. These results provide new insights into the ecology of Lake Superior coho salmon and may have important implications on the management of Great Lakes populations.

COMPARATIVE ANALYSIS OF METHODS FOR THE DETERMINATION OF NITRATE AND PHOSPHATE IN THE LAKES OF ST. JOHN'S UNIVERSITY

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Due to fertilizer runoff and wastewater, lakes on St. John's University campus have an overabundant growth of algae each year. When lakes experience an abundance of algae growth due to increased nitrate and phosphate levels, eutrophication occurs. This process results in consumption of dissolved oxygen and eliminates most aquatic life. To determine how much nitrate and phosphate are being introduced, standard methods for these analyses were employed. For phosphate determination, a spectrophotometric method using ammonium molybdate and tin (II) chloride was used. For nitrate, two methods were compared: a spectrophotometric cadmium reduction, and a W absorbance after acidification, with the cadmium reduction method giving more precise results. These nutrients are monitored over an extended period of time to develop a sound ecological land management system at St. John's University.

PURIFICATION AND CHARACTERIZATION OF AN ANTIFUNGAL COMPOUND EXTRACTED FROM A FUNGAL SOURCE

Ryan J. Jense, Dr. Katie J. Graham, Department of Chemistry, St. Johns University, Collegeville, Minnesota 56374*

Medical advancements and a continual growth of infectious diseases have caused an increase in immunocompromised individuals making fungal infections a more prevalent threat. The few antifungal drugs on the market today either produce adverse side effects or are ineffective because of emerging fungal resistance. New potent, nontoxic, antifungal drugs are needed. Fungal sources are logical providers of antifungal compounds since competition occurs between fungal species in most environments. Thus, the goal is to attempt to discover novel antifungal compounds from fungal sources.

KG 146A, an endophyte isolated from *Ceratiola ericoides* in Archbold Biological Preserve in Florida, was placed into a loaffled flask and shaken at 200 rpm in Sabouraud Dextrose broth for eight days. The media was extracted and partially purified using bioassay-guided fractionation. Disc diffusion bioassays were used to follow the activity; the test organisms were *Saccharomyces cerevisiae*, *C. albicans* 109, and *C. albicans* 406. Further use of HPLC techniques will yield the active compound. The antifungal compound will then be characterized by NMR, mass spectrophotometry, and other analytical tools. KG 146A appears to be a potential source for a new antifungal drug.

CO-LOCALIZATION OF CGRP AND NGF IN LAMINAE I & II OF MOUSE AND RAT SPINAL CORD DURING CARRAGEENAN INDUCED INFLAMMATION

Erik Johnson

Little is understood about the mechanisms behind chronic pain though its effects are often profound. Inflammatory pain is often mediated by hyperalgesia, an increased sensitivity to pain, but this pain is generally not continual and is more thought of as tenderness of the effected area. Peripheral administration of nerve growth factor (NGF) causes local inflammation but also affects neuropeptide concentrations in dorsal horn spinal cord neurons (Amann 1995, McMahon 1996). Concentrations of calcitonin gene related peptide (CGRP), a neuropeptide involved in spinal nociceptive processing, have been shown to increase in laminae I & II_o in response to such NGF administration. It has recently been shown that the trk A receptor (specific binding NGF receptor) and CGRP are co-localized in these spinal cord neurons

(Averill 1995). However, it is unknown whether NGF directly causes the CGRP increase or whether there is a change during the inflammatory process. Using the Carrageenan model of inflammation, mouse spinal cords were taken 0.5, 4, 24, and 48 hours post-bilateral intraplantar injection of Carrageenan in the hindpaw. CGRP and trk A were co-localized in the dorsal horn neurons using double labeling immunohistochemistry and graphic overlay imaging. Electron microscopy was also used to investigate the colocalization of CGRP and trk A in the vesicles of these neurons.

ANALYSIS OF HUMAN BLOOD DONOR SAMPLES TO DETERMINE CYTOTOXICITY TOWARD PORCINE AORTIC ENDOTHELIAL CELLS.

Jason Johnson, Hamline University, 1536 Hewitt Ave., St. Paul, MN 55104

Xenotransplantation is the transplant of organs, tissues, or cells from one animal species to another and is being looked upon as a potential solution to a severe lack of suitable allografts for patients in need of an organ transplant. Rejection of xenografts by the body's immune system is the major obstacle for the progress of xenotransplantation, though. The focus of this research was to investigate various human blood samples for their cytotoxicity toward porcine aortic endothelial cells as a model for xenograft rejection. Correlations were determined for levels of IgG and IgM type antibodies as compared to cytotoxicity of human blood serum samples. It is argued that if mechanisms controlling the levels of various classes of antibodies can be manipulated, xenograft rejection could be controlled similarly.

FUNCTIONAL CHARACTERIZATION OF THE VISUAL ENTRAINMENT PATHWAY FOR THE MOUSE CIRCADIAN PACEMAKER

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We are characterizing the sensitivity of the visual entrainment pathway for the mouse circadian system (C57BL/6J) by measuring its responsiveness to single and multiple light pulses of several durations. A behavioral assay will be used to measure light-induced resetting (phase shifts) of the circadian rhythm of running activity. Mice are initially entrained to a 12:12 light cycle and then placed into cages equipped to monitor activity using a data acquisition system (Chronobiology Kit, Stanford). Mice are then transferred to darkness to allow expression of each animal's circadian activity rhythm (driven by an endogenous neural circadian pacemaker). To determine the sensitivity to light the mice are individually exposed to either one 15-minute light pulse (white light, 5000 lux), multiple 15-minute pulses separated by durations of darkness (1-15 minutes), or a single light pulse (durations from 30s - 6h). All stimuli will be delivered at a time in the mouse circadian cycle that induces phase delays (a resetting to an earlier circadian time). As the photic entrainment pathway has been shown to integrate inputs over extended durations, we hypothesize that both multiple and very long duration stimuli will be interpreted by the pacemaker as a single stimulus. On the other hand, previous studies were performed in the phase advance region of the circadian cycle while our studies are being carried out at a time that induces phase delays. It is unknown whether delaying shifts can be followed by a second response during the same circadian cycle.

THE CORRELATION BETWEEN MICROANEURYSMS AND INTERSTITIAL FIBROSIS IN THE HYPERTENSIVE DIABETIC RAT HEART

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Previous studies concerning the cardiomyopathy of diabetic hypertensive rats have suggested that there may be a relationship between the number of microaneurysms and the

amount of interstitial fibrosis within the myocardium. To further examine that relationship, further studies were performed to ascertain whether or not the formation of microaneurysms and interstitial fibrosis might be correlated. Diabetes mellitus and hypertension were induced in the experimental group of Sprague-Dawley rats, while no physiological changes were induced in the control group. Diabetes mellitus was induced using 55mg/kg of alloxan monohydrate. The right renal artery of the experimental rats was banded to induce hypertension. The disease state was allowed to progress for 23 weeks, during which time the weight and plasma glucose levels were monitored. Before sacrificing the animals, latex was perfused into the heart to allow for later visualization of the microvasculature. After sacrificing the animals, the hearts were removed, fixed and prepared for both sectioning and light illumination. Finally, the number of microaneurysms were quantified and compared to the amount of interstitial fibrosis found within selected areas of the myocardium.

IN-VITRO EFFECTS OF BETULIN AND BETULINIC ACID ON HSV-1 AND HSV-2 USING VERO CELLS

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The utilization of plant derived chemicals has played a crucial role in the war against infectious agents. Betulin, a pentacyclic triterpene, is extracted from the outer bark of birch trees; betulinic acid is the carboxylated form of betulin. Currently the most effective drug used to treat Herpes Simplex Virus Type I (HSV-1) and Type 2 (HSV-2) is Acyclovir (ACV). However, ACV has multiple side effects and strains of HSV-1 and HSV-2 have developed mutants with resistance to ACV. I studied the effect of botulin and betulinic acid has against HSV-1 and HSV-2 in African green monkey kidney cells (Vero). Quantitatively, no plaque formation was observed in Vero cells treated with botulin at a concentration of 31 ng/ml compared to cells treated with ACV at a concentration of 3125 ng/ml using a viral concentration of 10 plaque forming units per well. The results indicate botulin is more effective than ACV in complete protection of cells against HSV-1 and HSV-2 infection. These findings may be useful in furthering the development of botulin as a new treatment for HSV-1 and HSV-2.

USING ANTISENSE OLIGODEOXYNUCLEOTIDES TO DECREASE NA⁺ - H⁺ ANTIporter ACTIVITY IN NEONATAL CARDIAC VENTRICULAR MYOCYTES.

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The Na⁺ - H⁺ antiporter has been demonstrated to play a key role in the regulation of intracellular pH in cardiac ventricular muscle cells. The antiporter is a Na⁺-dependent, amiloride-sensitive, proton extrusion mechanism which is activated by an increase in intracellular H⁺ concentration. An antisense oligodeoxynucleotide based on the initial coding sequence of the NHE-1 isoform of the Na⁺ - H⁺ antiporter was used to study the effects of decreasing expression of this protein on pH regulation in neonatal cardiac ventricular myocytes. Ventricular myocytes from 1-5 day old rat pups were maintained in culture for 24 to 36 hours prior to treatment with lipofectin and antisense oligodeoxynucleotide. Cultured cells were treated with 20 µg/ml lipofectin and 0.2 µmol/liter antisense oligodeoxynucleotide for 8 hours. Antisense treatment had no impact on Na⁺ - H⁺ antiporter activity in the initial 24 hours following transfection. Na⁺ - H⁺ Antiporter activity measured at 48 and 72 hours following transfection showed a marked decrease in the rate of recovery from an intracellular acid load as compared to lipofectin and untreated controls demonstrating a reduced expression of the Na⁺ - H⁺ antiporter.

DETERMINING THE EFFECT OF NUCLEAR PLOIDY ON REPRODUCTIVE SUCCESS IN *TETRAHYMENA THERMOPHILA*

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Tetrahymena thermophila, a freshwater unicellular ciliate, is capable of sexual reproduction through a process called conjugation. Some cell lines are more successful at conjugation than the others. We investigated the relationship of micronuclear ploidy on conjugal success by mating cells of abnormal numbers of chromosomes to a normal diploid partner. The results indicated that micronuclear behavior during and after meiosis appeared normal despite the loss of one, two and even three of the five pairs of chromosomes. Curiously, cells with multiple copies of a single chromosome in their micronuclei behave normally. We discuss a model in which successful post-meiotic micronuclear behavior depends on the numbers of kinetochores rather than chromosome constitution.

INVESTIGATING A TROPICAL FISH DISEASE

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The impetus for this inquiry came from a concern for the health of my captive-bred ornamental fish. The fish belong to the Cichlidae family and originate from Lake Malawi in equatorial Africa. Under certain conditions these Cichlids become inactive, stop eating, and exhibit abdominal distention. Death soon follows. The disease is mysterious because certain genera appear to be more susceptible and it strikes animals kept in a clean environment and fed a proper diet.

Two groups of fish were placed in separate 200-liter tanks. The first group was *Pseudotropheus* sp., a more susceptible genus. These fish are territorial and aggressive. The second group was *Scianochromis ahli*. These fish are also aggressive but loosely territorial. The fish were observed over an eight-month period. Within the first month some of the *Pseudotropheus* sp. bloated and died. The second tank had deaths relating to combat, but no fish became bloated. Dead and dying fish were sectioned for tissue samples and isolation of any potential pathogen. Pure cultures of a known fish pathogen, *Aeromonas* sp., were isolated. Live healthy fish were inoculated with this microorganism to prove its pathogenicity. If *Aeromonas* sp. is a common resident of freshwater environments why were not all of the fish affected? It appears that another factor was implicated and further research may point to the immune system of these attractive fish.

A 400-YEAR HISTORY OF AMHERST GLACIER, COLLEGE FJORD, ALASKA: THE RECORD FROM TREE RINGS AND HISTORICAL OBSERVATION

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In the last few decades global climate change has become an increasingly important field of study. One indicator of past climate variation is preserved in geologic records of glacier fluctuations. Ice retreat over the past century at Amherst Glacier, College Fjord, Alaska, has revealed a collection of glacially overrun trees near its recent terminal moraine. Crossdating ring-width series from these logs with regional master tree-ring chronologies of the western Prince William Sound area suggests that the glacier was advancing near this recent maximum about AD 1633, a pattern shared with other glaciers from the region. According to a retreat rate estimated from historical observations made from AD 1914-1957, ice began to recede from the terminal moraine about AD 1870. General retreat with minor readvances or standstills has

dominated Amherst Glacier over the past 125 years. Observations in 1971 and 1997 indicate that the rate of retreat was increasing, partially due to iceberg calving into a proglacial lake. This research raises two interesting unanswered questions: (1) What climatic inputs in the early-middle seventeenth century led to the advance of the glaciers in the Prince William Sound region, and (2) Is the recent increase in the rate of retreat of Amherst Glacier due to anthropogenic influences or natural climatic cycles?

COLOCALIZATION OF THE TRK A RECEPTOR AND SUBSTANCE P IN THE INFLAMED MOUSE SPINAL CORD

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During the inflammatory response, there is an increase in substance P in the dorsal horn of the spinal cord. The trophic factor, nerve growth factor which binds to the Trk A receptor, is thought to play an integral part in this process. Studies in our lab have confirmed that there is an increase in Trk A receptors, following Carrageenan inflammation in the mouse. The addition of nerve growth factor to superfused spinal cord has resulted in an increased content of substance P. so therefore we hypothesize that nerve growth factor is able to directly cause the release of substance P during inflammation. Using spinal cord tissue from inflamed mice at time points of 30 minutes, 4 hours, 24 hours and 48 hours post bilateral intraplantar Carrageenan treatment, immunohistochemical techniques were used to colocalize the Trk A receptor with substance P in lamina I and II of the dorsal horn of the spinal cord. Nerve Graphic overlay of images will be used to visualize labeled Trk A receptors and substance P. If the labeling patterns using immunohistochemistry are identical, this would strengthen the hypothesis that nerve growth factor directly causes the release of substance P during inflammation. Electron microscopy techniques will also be utilized to investigate if the labeling occurs inside the vesicles.

REGULATION MUTANTS OF MAIZE RECOMBINANT

C₄-PYRUVATE, Pi DIKINASE REGULATION IMPAIR CATALYSIS

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A key enzyme of the C₄-photosynthetic pathway in higher plants is pyruvate, orthophosphate dikinase (PPDK). As a pivotal enzyme in the C₄ pathway, it undergoes diurnal light-dark regulation of activity which is mediated by a single bifunctional regulatory protein (RP). RP specifically inactivates PPDK in the dark by an ADP-dependent phosphorylation of an active-site Thr residue (Thr-456 in maize). Conversely, RP activates inactive PPDK in the light by phosphorolytic dephosphorylation of this same Thr-P residue. We have cloned the maize C₄-PPDK gene into an expression vector for producing recombinant PPDK in *E. coli* for site-directed mutagenesis studies of the active-site regulatory Thr residue. Preliminary analysis of three of these mutants (Thr456Arg, Thr456Tyr, and Thr456Asp) shows these substitutions either lower or abolish enzyme activity. Presumably, in the case of the aspartate substitution, occurs because the negative charge introduced via the aspartate carboxyl group into the active-site functionally mimics the RP inactivation/phosphorylation at this position. For the other mutants, two other mechanisms may be operating to effect catalysis. Further discussion of these mechanisms and extensive data concerning in vitro inactivation/phosphorylation of these mutant enzymes as effected by their respective substitutions will be presented.

PRECONDITIONING-INDUCED ATTENUATION OF PURINE METABOLITE AND ACCUMULATION DURING ISCHEMIA (ISC): MEMORY AND MULTIPLE CYCLES

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Brief myocardial ISC is known to attenuate purine metabolite accumulation in the interstitial fluid (ISF) during subsequent

ISC. We determined how this attenuated purine accumulation was altered by: 1) extended reperfusion (REP), and 2) multiple cycles of brief ISC. Microdialysis probes were used to assess ISF levels of the purine metabolites adenosine, inosine, and hypoxanthine. In one series of experiments, two 10 min periods of regional ISC were separated by 10 (n=6), 60 (n=6), or 180 (n=6) min of REP. In the 10, 60, and 180 min REP groups the increase in ISF purine metabolites during the second ISC was 47%, 55%, and 53% of that seen during the first ISC, respectively. In a second series of experiments, hearts were exposed to 120 min of ISC with or without 5 preceding cycles of transient ISC (10 min ISC; 10 min of REP). The increase in ISF purine metabolites during the multiple cycles of ISC was progressively attenuated; by the fifth ISC the increase was only 20% of that seen during the first ISC. Following repeated ISC, there was only a minimal increase in ISF purine metabolites during the first 20 min of the 120 min ISC. However, ISF purine metabolites eventually increased to approximately 90% of that seen during 120 min of ISC in the group without preceding ISC. These data imply that attenuated purine metabolite accumulation is progressively greater with multiple cycles of brief ISC and has a memory time of at least 180 min. However, this attenuation is likely not due to ATP depletion since ISF purine metabolite levels eventually increase during prolonged ISC.

EXOGENOUS ADENOSINE DOES NOT PREVENT ATTENUATED PURINE METABOLITE PRODUCTION DURING REPEATED ISCHEMIA (ISC)

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Brief transient ISC decreases the accumulation of purine metabolites in the interstitial fluid (ISF) during a subsequent period of ISC. Dual cardiac microdialysis was used to determine if the attenuation of ISF purine metabolite accumulation during the second period of ISC exists after supplying the heart with adenosine (ADO) which was lost following the first period of ISC. Two microdialysis probes were implanted in the myocardium perfused by a coronary artery. Microdialysis probes were used to assess myocardial ISF levels of ADO, inosine (INO), and hypoxanthine (HYPO) as well as to deliver ADO locally to the surrounding tissue. Rabbits (n=5) were exposed to 10 min of regional myocardial ISC, 60 min of REP and a second 10 min period of ISC. One probe was perfused with Krebs-Henseleit buffer (KHB) throughout the entire protocol. The other probe was perfused with KHB containing 1 mM ADO from 10-40 min of the 60 min REP period; KHB without ADO was perfused through the probe at all other times. Similar increases in dialysate purine metabolites during the first period of ISC were observed in both probes. ISF purine metabolites increased with administered ADO during REP and then decreased to levels similar to those in the other probe upon cessation of local ADO administration. These data suggest that supplying ADO to the myocardium after an initial period of ISC does not prevent attenuated purine production during a second period of ISC. *Supported by NIH HL-46027.*

NON-DESTRUCTIVE EVALUATION OF ADVANCED COMPOSITE MATERIALS

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Technological and manufacturing improvements of Advanced Composite Materials are currently being developed for a wide range of uses in the aerospace industry and for defense applications. Research currently being conducted at the University of Wisconsin - La Crosse is aimed at developing a

testing procedure for composite materials which will be able to predict internal defects. Due to manufacturing technologies, it is inevitable that defects will get into composite samples. Computer software is being developed to predict internal defects by calculating the scattered wavefield of elastic waves. This wavefield is achieved mathematically by using the T-matrix method. Upon completion of the program, the type and distribution of defects in Advanced Composite Materials will be classified. In the mean time, experiments are being conducted on composite samples with controlled variation of porosity. Air-coupled Acoustic NDE methods are being explored to study the presence and effects of defects within composite materials.

GENETICALLY ENGINEERING A HISTIDINE AFFINITY PURIFICATION TAG ON MAIZE RECOMBINANT C₄-PYRUVATE,PI DIKINASE FOR X-RAY CRYSTALLOGRAPHY STUDIES

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A key enzyme of the C₄-photosynthetic pathway in higher plants is pyruvate, orthophosphate dikinase (PPDK). Studies show that it controls the photosynthesis rate in maize leaves. Hence, one strategy for improving yields in C₄ crop species is to improve photosynthetic performance of PPDK using genetic engineering technology. We have made the first step towards this goal by cloning the PPDK gene from maize into the bacterium *E. coli*. When cloned in this bacterium, it can be selectively changed or altered using recombinant DNA techniques. However, before this enzyme can be altered in a useful way, we need to have a detailed picture of how the enzyme is put together. This can be accomplished by obtaining the crystal structure for tetrameric maize C₄ PPDK using X-ray crystallography. For this technique to be successful, large amounts of highly pure enzyme need to be obtained. To achieve this level of purity and yield, we have genetically engineered a histidine tag onto the C-terminal end of the enzyme for affinity purification using nickel-agarose chromatography. A discussion of the engineering strategy used as well as data on enzyme purification will be presented.

GENERATION OF NEW ADH TRANSFORMED LINES BY A Δ2-3 MOBILIZATION OF ADH INSERTS IN DROSOPHILA

MELANOGASTER

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Position effects, alterations in gene expression arising from the location of a gene, often modify to expression of transgenes inserted into random locations in the genome. The objective of this study was to generate and isolate new alcohol dehydrogenase (Adh) transformed lines. A Δ2-3 mobilization method was used to induce a transposon containing the alcohol dehydrogenase gene of *Drosophila melanogaster* to move to an autosomal location. Seventy new transformed lines were established by selecting on 6% ethanol. These lines revealed a variety of different patterns of larval Adh expression, some of which have not been observed before. Each abnormal pattern is stable and is characterized by the absence, presence or reduction of Adh expression in certain tissues. Possible molecular mechanisms for this abnormal gene expression will be discussed.