ABSTRACTS OF PAPERS PRESENTED AT THE 2004 COLLEGIATE MEETINGS

EASTERN REGION

PELLISSIPPI STATE TECHNICAL COMMUNITY COLLEGE
KNOXVILLE, TENNESSEE

TRANSFORAMINAL LUMBAR INTERBODY FUSION: A RETROSPECTIVE STUDY OF LONG-TERM PAIN RELIEF AND FUSION OUTCOMES. ***Cody A. Chastain and Scott Hodges,*** *Southern Adventist University, Collegedale, Tennessee and Center for Sports Medicine, Chattanooga, Tennessee.* This retrospective study examined the postoperative pain, disability levels, and fusion status of Transforaminal Lumbar Interbody Fusion (TLIF) patients after at least 4 years to establish a long-term precedent for physician expectations. Forty-two patients participated in this study. The average patient's postoperative pain was lowered by 2.4 points on a 0–10 scale. The average patient's perceived disability, as evaluated by the Oswetry Pain Index, decreased by 10.5%. Twenty-five of 39 patients evaluated by X-ray films showed spinal fusion in their operative vertebral levels, and no movement was detected in the operative vertebral levels of those 39 patients. Patients with no fusion and no movement exhibited postoperative pain levels 1 point greater than patients with fusion. The postoperative disability scores were greater by only 1.7%. Thirty-six of the 42 patients would elect to have the surgery again for a 85.7% approval rating. In conclusion, TLIF is an effective method of alleviating intractable back pain over an extended time period.

2004 Eastern Region Collegiate Meeting participants.

AN ANALYSIS ON THE COMMON TICK SPECIES FOUND IN THE CHATTANOOGA-HAMILTON REGION OF TENNESSEE AND THEIR CORRELATION TO THE REPORTED CASES OF VECTOR-BORNE DISEASES FROM 1995–2001. ***Astrid von Walter and Safawo Gullo,*** *Southern Adventist University, Collegedale, Tennessee. Amblyomma americanum, Dermacentor variabilis, Ixodes scapularis,* and *Rhipicephalus sanguineus* are three-host ticks commonly found in the Chattanooga-Hamilton region of Tennessee. The first three genera are medically important because they transmit Ehrlichiosis or Rocky Mountain spotted fever, and Lyme disease, respectively. *Rhipicephalus* is not known to vector any human infecting pathogens; therefore, not considered clinically significant. Certain anatomical features of hard ticks such as capitulum and scutum ornamentation are used to differentiate between genus and species. The focus of this study was to correlate the frequency of *A. americanum, D. variabilis,* and *I. scapularis* in the Chattanooga region to reported cases of tick-borne diseases from 1995 through 2001. *D. variabilis* showed a direct relationship between its frequency and the reported cases of Rocky Mountain Spotted Fever. However *I. scapularis* was not as prevalent as *A. americanum* but there were still more cases of Lyme disease.

MODELING THE STRUCTURE OF THE NUCLEOSOME AT 2.5 ANGSTROM RESOLUTION. ***Merri Loy,*** *Pellissippi State Technical Community College, Knoxville, Tennessee.* Research has shown students develop analytical thinking skills better, and are able to grasp abstract concepts more easily, when hands-on materials are employed in the classroom. This project utilizes the structure elucidated by Dr. G. Bunick (Univ. Tennessee, Knoxville) to build a 3-D scale model of the nucleosome core particle with associated DNA, illustrating several key features of the structure. Each histone has a characteristic histone fold that is involved in forming histone handshakes between heterogeneous dimers (H2A/H2B and H3/H4). Arginine residues in each of the histones interact with the DNA minor groove, distorting the helix so that it wraps around the core particle. The DNA puts stress on the histone structure, causing the alpha helices to bend, producing asymmetries in the core particle. The histone tails of adjacent particles associate in an unexpected manner in the crystal structure. The completed model will be used in the undergraduate classroom.

DETECTION OF METHANOL MASERS IN W49A. ***Lisa M. Tackett,*** *Roane State Community College, Harriman, Tennessee.* Methanol masers have been detected using the Haystack 37-meter Radio Telescope at the Massachusetts Institute of Technology. Methanol masers have been recognized as a possible signpost for massive star formations. The project involves a search in the CO outflow region of W49A (in Aquilia); we are searching for Class I or II, methanol masers. The first portion of the search is conducted at a frequency of 44 GHz by mapping the surrounding areas until a maser is detected. After a methanol maser has been detected at the 44 GHz frequency additional maps may be preformed at the transitional frequency lines. These are normally run around 25 GHz and 36 GHz. By comparing the different frequency maps the excitations of the maser may be discovered.

A PREMLIMINARY COMPARISON OF *PAGURUS SAMUELIS* (DECAPODA: ANOMURA) POPULATIONS FROM NORTH AND SOUTH MONTEREY BAY, CALIFORNIA.

April D. Sjoboen and Stephen G. Dunbar, *Southern Adventist University, Collegedale, Tennessee, and Loma Linda University, Loma Linda, California.* Morphometrics, including shield length, wet weight, sex, shell species and shell weight, were collected on *Pagurus samuelis* collected at Monterey Bay, California in July 2003. Empty gastropod shells and individuals of each snail species were counted at each site to give a profile of relative abundances for those shells potentially available to hermit crab populations. Although the ranges of hermit crab sizes and body wet weights were approximately equal over both sites, there was a significant difference in the mean size of hermit crabs between sites. Santa Cruz males constituted the largest crabs but did not outnumber the females. At Carmel males were both the largest crabs found and more numerous than all females combined. The range of shell species used by *P. samuelis* was outside the range of snail species collected, suggesting that shells are being brought into local populations of hermit crabs by either inshore wave action or immigrant individuals.

TRACTOR BEAMS AND BACTERIA. ***Stanley Allen and Chris Hansen,*** *Southern Adventist University, Collegedale, Tennessee.* This talk will report on undergraduate research in optical trapping with hopeful applications to bacterial study. We will follow-up on earlier research that included basic apparatus assembly and attempted trapping. Since our last report, we have improved our apparatus and reproducibly trapped small spheres in solution. Calculations of our trapping force are encouraging and we hope to soon trap and study bacteria.

ERGOSTEROL DEPLETION LEADS TO REDUCED STE2P ACTIVITY IN *SACCHAROMYCES CEREVISEAE.* ***Nicole Billings and Amanda Dalton,*** *Tennessee Wesleyan College, Athens, Tennessee.* Ste2p is a highly characterized G-protein coupled receptor that mediates mating between haploid yeast cells. Receptor activation initiates a signal transduction pathway leading to nuclear fusion between yeast cells of opposing mating types. In this study, we have looked at the function of ergosterol in Ste2p activity. Ergosterol levels were reduced by treating cells with ketoconazole, an azole antifungal agent known to inhibit oxisterol synthesis. Previous studies indicated that reduction of ergosterol levels changed Ste2p activity, resulting in reduced binding capacity for the pheromone and increased growth inhibition after exposure to pheromone. In this study, we observed that ergosterol depletion significantly reduced mating projection (shmoo) formation. In addition, proteolytic cleavage of Ste2p in ergosterol-depleted cells indicated a conformational change in the receptor, offering an explanation for altered receptor function.

MIDDLE REGION
TENNESSEE STATE UNIVERSITY
NASHVILLE, TENNESSEE

INVESTIGATIONS OF ANTI-CANCER COMPOUNDS FROM ROOT EXTRACTS OF *ECHINACEA.* ***Lakeshia N. Wright, Leslie Buggs, Amber Collins, Ugoshi Ike, Todd Gary and E. Lewis Myles,*** *Tennessee State University, Nashville, Tennessee.* Many compounds found in plants have anti-bacterial, anti-fungal, and anti-cancer activities. Over 25% of our common medicines contain at least some compounds obtained from plants. In the United States approximately 10% of our major drugs have plant extracts as their active ingredient. In less developed countries the World Health Organization estimates that 75–80% of the people rely on plant-based medicines for primary health care. The herb *Echinacea purpurea* (purple coneflower) and *Echinacea pallidae* produces natural compounds that may increase the efficiency of mammalian immune systems. This investigation used extracts from the dried roots of *Echinacea* on a breast cancer cell line, BT549, to determine possible anti-cancer activity. Cells were grown at 37°C with 5% CO_2 and exposed to 7, 14 or 21 mL of DMSO or the extract. The extract was obtained by conventional methods and dissolved in DMSO. The growth rates of cells in the control and experimental groups were significantly different in that both plants showed a reduction in growth. The root extract appears to inhibit growth of cancer cell line BT549. *E. pallidae* reduced the growth of cell line BT549 more significantly that *E. purpurea.* These results indicate that further studies are necessary to identify anti-carcinogenic compounds in *Echinacea.* (Supported by USDA/CSREES Evans-Allen Funds and MARC Grant # 5T34MO7663)

2004 Middle Region Collegiate Meeting participants.

THE EXPRESSION OF C/EBP ALPHA AND C/EBP BETA IN THE CANCER CELL LINE BT549 AFTER EXPOSURE TO THE PLANT EXTRACT OF *HYPERICUM ANTHOS.* ***Alicia Cleveland,*** *Tennessee State University, Nashville, Tennessee.* The mutation of the gene C/EBP results in a mutant protein that affects the anatomy and physiology of an organism. The gene causes the mammary gland to overexpress and or be constantly activated to induce a proliferation of cells within the breast, which leads to breast cancer. The C/EBP protein is a transcription factor that regulates metabolism, energy partitioning, fat development, and feed and energy efficiency through the presence of activated Ras and the Ras signaling pathway. The C/EBP protein is typically found in cancer cells that become anchorage independent, metastatic, and gain invasive. Our lab is studying the growth patterns of breast cancer cell line BT549. Moreover, these studies have shown that when exposed to the crude extract from *Hypericum anthos* at low concentrations, cell growth is inhibited and cells die. *Hypericum anthos* has as its active ingredient hypericin, which induces apoptosis. Hypericin also may induce an invasive metastatic cancer cell, by working synergistically with the protein C/EBP protein. To analyze these activities in the BT549 cell line, cell viability was assessed using Trypan Blue and cell proteins

were compared by electrophoretic analysis. The results show that the cells lost their anchorage density dependency and were still alive due to synergistic effects, possibly of the two components, C/EBP protein and *Hypericum anthos*. Studying cancer cells will help to identify antagonistic compounds that inhibit metastatic invasive cancer cells, and provide a potential treatment for breast cancer.

IMMUNOTOXIC EFFECT OF PENTACHLOROPHENOL ON HUMAN NATURAL KILLER CELLS CAN BE DIMINISHED BY NK-STIMULATORY INTERLEUKINS. ***Telpriore Tucker, Adrian Reed, Bommanna Loganathan, and Margaret Whalen,*** *Tennessee State University, Nashville, Tennessee.* Pentachlorophenol (PCP) is used predominantly as a fungicide in wood preservatives. PCP also has been used as a bactericide and fungicide to protect many products such as adhesives, paper, paints, and textiles. Natural killer (NK) lymphocytes are central in immune defense against virus infection and tumor formation. This study investigated the effects of a range of PCP concentrations on the tumor-lysing function of human NK cells. NK cells were exposed to 10, 5, 2.5, 1, 0.5, 0.25, and 0.1 mM PCP for 1, 24, or 48 h and 6 days. A 1 h exposure to 10 mM PCP caused a 70% decrease in lytic function. Exposure to 10 mM PCP for 24 or 48 h, and 6 days decreased cytotoxic function by 89%, 99%, and 98%, respectively. NK cells exposed to 5 mM PCP showed decreases in lytic function that were comparable to those seen at 10 mM with the exception of the 1h exposure. Exposure of NK cells to as little as 0.5 mM PCP for 48 h or 6 days decreased lytic function about 50%. Pretreatment of highly purified NK cells with the NK-stimulatory interleukins (IL) 2 and 12 was carried out to determine if they could protect NK cells from the PCP-induced loss of tumor-lysing function. The pretreatment of NK cells with IL-2, IL-12, or IL-2 + IL-12 for 24 h or 5 days significantly diminished the decrease in tumor lysis caused by a 24 h exposure to 2.5 mM PCP.

EFFECTS OF STRESS ON DOPAMINE 2 RECEPTORS AND ALCOHOL SELF-ADMINISTRATION IN RATS. ***Theresa Tholkes and Nick Ragsdale,*** *Belmont University, Nashville, Tennessee.* Recent work has shown that an organism's environment plays a role in the concentration of dopamine receptors located within the cerebrum. Also, previous investigators have established the correlation between stressful life events and an increase in addictive behavior. Finally, dopamine receptors may have a role in addictive behavior. The purpose of this study was to determine the effect of stress on the dopamine receptor levels in the prefrontal cortex of juvenile male rats. Rats were subjected to swim stress test for 30, 45, and 60 minutes, the pre-frontal cortex was harvested, and the proteins were analyzed by Western immuno-blotting. Additionally, this study will investigate the correlation between the levels of prefrontal cortex dopamine receptors and the self-administration of alcohol following a swim stress test. Preliminary results indicate that rats subjected to increasing levels of stress have decreasing levels of dopamine receptors in the prefrontal cortex. Increased stressful events in life may lead to increased addictive behavior due to low dopamine receptor levels.

EPIDERMAL GROWTH FACTOR STIMULATED CELL MIGRATION IN COLON EPITHELIAL CELLS REQUIRES SRC-DEPENDENT p38 ACTIVATION. ***Anastasia Golovin, Mark R. Frey, and D. Brent Polk,*** *Vanderbilt University Medical Center, Nashville, Tennessee.* The ability of intestinal epithelial cells (IEC) to migrate into regions denuded of epithelium and close a wound is critical for maintenance of a healthy gastrointestinal tract. However, the signaling pathways involved in IEC migration are poorly understood. Our laboratory has demonstrated that activation of epidermal growth factor (EGF) receptor (R) accelerates IEC migration, suggesting a role for EGF-stimulated signaling in intestinal wound healing. Furthermore, protein kinase C (PKC), AKT/PKB, and Src are required for EGF-stimulated migration of young adult mouse colon (YAMC) cells. More recently, we have identified the p38 mitogen-activated protein kinase as a requisite mediator of this process. The current study seeks to determine, using signaling inhibitors and Western blot analysis for activated kinases, the molecular pathways regulating p38 activation downstream of EGFR. EGF-stimulated p38 activation in YAMC cells was inhibited by three different Src inhibitors (PP1, PP2, and CGP XXXX). In contrast, AKT and PKC inhibitors failed to abrogate EGFR-induced p38 activity. Furthermore, stimulation of PKC by phorbol ester failed to activate p38 in these cells. Taken together, these data demonstrate that EGF-stimulated IEC migration requires a Src-dependent, PKC- and AKT-independent activation of p38, and implicate p38 as a potential mediator of intestinal epithelial wound healing.

DETECTION OF THE MAJOR VAULT PROTEIN AND VAULT POLY ADP-RIBOSYL POLYMERASE IN HIGH- AND LOW-RISK HUMAN PAPILLOMAVIRUS CELLS. ***Melissa Fullerton and Jennifer Thomas,*** *Belmont University, Nashville, Tennessee.* Vaults, which are barrel shaped structures composed of three proteins and a small portion of RNA, have been implicated as nucleocytoplasmic transporters and mediators of chemotherapeutic multidrug resistance. Vault levels have not been studied in the context of cervical cancer. For this reason, this research examined whether or not vault protein levels, specifically, levels of the major vault protein (MVP) and the vault poly ADP-ribosyl polymerase protein (VPARP), are altered in human papillomavirus (HPV) cell lines, which mimic cervical cancer cells. Levels of proteins in HPV cell lines of both high-risk types, which are associated with cancer, and low-risk types, which are not, were examined. Additionally, protein levels of low-risk cell lines that were grown in methylcellulose, which causes the cells to differentiate and grow more like a tissue, were studied. Western analysis for p53 affirmed that all cell lines and lysates behaved as expected based on previous studies. The results from low-risk cell lines show a gradual increase in levels of expression of MVP with increasing exposure to methylcellulose. These results are somewhat surprising in that there are detectable levels of a protein associated with cancer in a cell line not associated with cancer. The results suggest that as the cell growth and development becomes more similar to tissue, the levels of the major vault protein increase. The results from VPARP reveal possible breakdown products in cell lines expressing high-risk HPV oncoprotein E7.

ANALYSIS OF VIOLACEIN ISOLATED FROM *ALTEROMONAS LUTEOVIOLACEA*. ***Jessica Bekar, Jon Lowrance, Kent Clinger, and William Tallon,*** *Lipscomb University, Nashville, Tennessee.* The marine bacteria *Alteromonas luteoviolacea* produces a violet pigment known as violacein. Violacein was isolated by extraction with acetone and purified using thin layer chromatography, macroscale distillation, and alumina column chromatography. The violacein was analyzed by UV/Visible

Spectrophotometry and Time of Flight Mass Spectroscopy. The HOMO, LUMO, conformational energy, and electrostatic potential were analyzed using the software program Hyper Chem. Had crystals been obtained, they would have been analyzed by X-Ray Diffraction to determine the crystal structure.

THE EFFECT OF LOW DOSES OF 4,4'-BIPYRIDIN-1-IUM BROMIDE MONOHYDRATE ON LIPID PEROXIDATION IN HUMAN MONONUCLEAR PROGENITOR CELLS (U937 CELLS). ***Tionanatasha K. Newell, Brooke Henderson, Justin Anderson, Peter Iyere, Lionnie Sharpe, Samuel E. Adunyah, and William Boadi,*** *Tennessee State University, Nashville, Tennessee (TKN, BH, JA, PI, LS, WB) and Meharry Medical College, Nashville, Tennessee (SEA).* Paraquat (1,1'-dimethyl-4,4'bipyridylium dichloride), one of the most widely used herbicides, is known for its severe toxicity to mammals, including humans. One explanation for the mechanism of paraquat toxicity is the concept of membrane-damage caused by lipid peroxidation mediated by superoxide anions. In our laboratory, a new compound, 4'4-bipyridin-1-ium bromide monohydrate, has been recently synthesized and reported. This compound is an analog of paraquat. No studies have yet been done on the toxicity of this compound. We hypothesized that the new compound can also induce lipid peroxidation in cell membranes. Studies on 4,4'bipyridin-1-ium bromide monohydrate, using human Mononuclear Progenitor Cells (U937) were conducted. Untreated and control cells were cultured side by side with U937 cells treated with various concentrations (0–180 M) of 4,4'-bipyridin-1-ium bromide monohydrate. Untreated cells did not contain any reagents used in the oxidation process. Control cells contained all reagents, except the different doses of the compound. Following incubation for 24 h at 37°C, the cells were pelleted by low speed centrifugation and lysed by repetitive freeze/thawing in distilled water (350 µl). Following centrifugation (15,000 g for 10 min) the supernatant, was used to measure the lipid peroxidation levels as described by CALBIOCHEM's Lipid Peroxidation Assay Kit (Cat. No. 437634). Briefly, 650 µL of diluted R1 (chromogenic reagent) were added to clean Eppendorf tubes. 200 µl of the cell sample were added and vortexed for 3–4 seconds. For the assay using MDA only, 150 µl of 12 N HCl were added. The samples were then incubated for one h at 45°C, and read in a plate reader at 540 nm. The levels of lipid peroxides were expressed as levels of malondialdehyde and calculated using a molar extinction coefficient of 0.1176 $M^{-1}cm^{-1}$. The results indicated that lipid peroxides increased in a dose-dependent manner for the doses tested. Thus, the above compound can cause peroxidation in cells, which could affect human health. Further studies will correlate lipid peroxides to glutathione levels in U937 cells.

WESTERN REGION
RHODES COLLEGE
MEMPHIS, TENNESSEE

THE EFFECT OF GLYCOSYLATION ON THE VIRULENCE OF INFLUENZA A (H3N2) VIRUSES. ***Kimberly C. Bartmess and Jon McCullers,*** *Rhodes College, Memphis, Tennessee and St. Jude Children's Research Hospital, Memphis, Tennessee.* Influenza is a major cause of death worldwide, and research into specific virulence factors is needed. The membrane glycoprotein hemagglutinin (HA) is important in influenza's infection cycle, and, because it is the main antigen of the virus, antigenic variation of the HA is favored in order to evade the body's immune system. Variation can include a change in the number of glycosylation sites. The goal of this project was to investigate the effect the degree of HA glycosylation has upon viral replication and virulence of influenza viruses of H3N2. Reassortant viruses that vary only in the HA and had 7, 9, and 12 glycosylation sites were created using reverse genetics. We characterized these viruses for replication and virulence in vivo and in vitro. We hypothesized that the degree of glycosylation of influenza viruses is inversely related to virulence in a naïve host due to glycosylation allowing easier clearance by collectins. In a study of virulence, mice infected with a virus that had an HA with low glycosylation (7 sites) lost significantly more weight than a virus with an HA with 12 sites. The mouse lethal dose was over one log greater for the more glycosylated virus. A potential explanation for the decreased virulence of highly glycosylated viruses is that greater levels of glycosylation allow for easier clearance by collectins.

AMPA-TYPE GLUTAMATE RECEPTOR SUBUNIT DISTRIBUTION IN OCULOMOTOR AND FACIAL MOTOR NUCLEUS IN RAT AND CHICKEN BRAIN. ***Cynthia Caceres, Kim Ries, Claudio A. B. Toledo, Raquel Pires, Malinda E. C. Fitzgerald and Anton Reiner,*** *Christian Brothers University, Memphis, Tennessee (KR, MECF), Universidade Cidade de Sao Paulo, Brazil (CABT, RP), and University of Tennessee, Memphis, Tennessee (CC, AR).* The distribution of the AMPA-type glutamate receptor (GluR) subunits was investigated in the oculomotor and facial motor neurons in brain sections from rats and chickens. Antibodies used included those directed against AMPA receptor subunits GluR1 and GluR4, and one antibody that detects both GluR2 and GluR3 subunits. Neuronal perikarya in the oculomotor nucleus of both rats and chickens were observed to be immunopositive for GluR2/3 and GluR4. In both species, the predominate subtype appeared to be GluR4. In the facial motor nucleus of the rat and chicken, both anti-GluR2/3 and anti-GluR4 labeled nerve cell bodies. Neither the oculomotor nor the facial motor neurons of the rat or chicken immunolabeled for GluR1. Our data indicate that part of the neuronal response of oculomotor and facial neurons to glutamate is mediated via AMPA-type glutamate receptors possessing some combination of GluR2, GluR3 and GluR4 subunits. The similarity observed between species in the types of subunits found in oculomotor and facial nuclei suggest an evolutionarily conservative role for glutamate transmission in the activation of these cranial motor nuclei. (Supported by NIH MIRT award (1T37TW00123–03, MECF), NIH EY-05298 (AR), and FAPESP 00/04536–2 (CABT))

PYROLYSIS GAS CHROMATOGRAPHY-MASS SPECTROMETRY AND TRANSMISSION ELECTRON MICROSCOPY IN THE CHARACTERIZATION OF ULTRAHIGH MOLECULAR WEIGHT POLYETHYLENE MICROSTRUCTURE. ***Richard D. Redfearn, Carl W. Carlson, Ann M. Viano, Karyn E. Spence, Matthew V. Shanks, and Asit K. Ray,*** *Rhodes College, Memphis, Tennessee (RDR, CWC, AMV, KES, MVS) and Christian Brothers University, Memphis, Tennessee (AKR).* Ultrahigh molecular weight polyethylene (UHMWPE) is currently the industry standard for use in large human joint prostheses. The combination of its relative nonreactivity in the body and its

mechanical properties make UHMWPE an excellent choice to replace the cartilage in total knee and hip replacements. However, as the prosthesis ages, the UHMWPE begins producing small wear particles, which cause many adverse biological reactions including osteolysis. Techniques such as gamma-irradiation-induced crosslinking have been developed to reduce the production of these wear particles. While the effects of these techniques have been studied using mechanical methods and Transmitting Electron Microscopy (TEM) and Scanning Electron Microscopy images, the effects these treatments have on the molecular structure are largely unknown. The molecular structure of UHMWPE is difficult to ascertain because of the inability of solvents to dissolve the extremely large polymer chains. To obtain an understanding of the molecular structure of UHMWPE, we used solid-state pyrolysis coupled with gas chromatography-mass spectrometry to separate and identify off-gases. We identified certain products as coming from reactions due to branching and crosslinking and used the relative abundance of each to compare the extent of crosslinking due to different treatments of UHMWPE. The data from the pyrolysis was related to data collected through TEM images.

DEVELOPMENT OF NEW CHEMOTHERAPY TREATMENT FOR RETINOBLASTOMA. ***Sandra Culpepper, Jay Blundon, and Michael Dyer,*** *Rhodes College, Memphis, Tennessee (SC, JB), and St. Jude Children's Research Hospital, Memphis, Tennessee (MD).* Retinoblastoma, a developmental childhood tumor of the retina, is the third most common form of cancer in infants. Depending on the severity, current treatment of retinoblastoma often involves enucleation of one or both eyes in order to prevent metastasis. Current chemotherapy treatments combined with laser and cryotherapy have been helpful in an attempt to salvage vision, but in approximately 40–50% of cases the cancer returns following treatment. To help preserve vision and quality of life, we began experimentation to develop a more effective method of chemotherapy treatment for children with retinoblastoma. Drugs chosen for treatment affect tumor cells via different apoptotic pathways, and included carboplatin, topotecan, and vincristine. We found significantly decreased tumor viability in cultured cell experiments, especially with combination drug treatments. Using the most optimal drug therapy determined by our cell culture experiments, we now show significant improvement in retinoblastoma therapy in in vivo mouse experiments.

USING AN EIGHT-ARM RADIAL MAZE TO STUDY LEARNING AND MEMORY IN MICE LACKING INTERLEUKIN-16. ***Karen E. Dobyns and Jay Blundon,*** *Rhodes College, Memphis, Tennessee.* Neuronal interleukin-16 (NIL-16) is a brain protein found in the hippocampus, an area important in learning and memory. The C-terminal half of NIL-16 is identical to pro-interleukin-16, the precursor to the cytokine IL-16. Caspase-3, the enzyme that cleaves IL-16 from NIL-16, and CD4, an IL-16 receptor, are also found in the hippocampus. We hypothesize therefore that IL-16 serves as a molecule that may influence neuronal signaling in the hippocampus, and thereby play a role in memory formation and/or memory retention. We compared spatial learning and memory retention in wild-type and IL-16 knockout mice using an eight arm radial maze. By placing food rewards in four of eight of the maze arms, we recorded errors in both short term memory (mice re-entered arms where the reward had just been eaten) and long term memory (mice entered arms that never contained food) during a two week period. One week later, mice were given a final run to test spatial memory retention. The groups did not vary significantly in memory errors until the final run one week after maze learning. The knock-out mice then showed significantly more short and long-term memory errors, suggesting that IL-16 is important in spatial memory retention.

INTRALYMPHOCYTE FREE MAGNESIUM AND CALCIUM IN RATS TREATED WITH ALDOSTERONE. ***Patrice M. Driscoll and Robert Ahokas,*** *Christian Brothers University, Memphis, Tennessee and University of Tennessee, Memphis, Tennessee.* Aldosterone interacts with intralymphocyte receptors and decreases the intracellular concentration of ionized magnesium ($[Mg^{2+}]_i$), yet such ion concentration changes have not ever been measured in single cells using flow cytometry. We measured intralymphocyte free magnesium ($[Mg^{2+}]_i$) using the molecular probe mag-fluo-4 and intralymphocyte free calcium ($[Ca^{2+}]_i$) using the molecular probe fluo-3 in 9 rats treated for four weeks with aldosterone, 5 rats treated for four weeks with aldosterone plus spironolactone, and 3 untreated control rats. $[Mg^{2+}]_i$ and $[Ca^{2+}]_i$ also were measured in lymphocytes incubated in vitro with aldosterone, utilizing a flow cytometry method. The data were analyzed by analysis of variance and significant differences between groups were determined using the Tukey HSD multiple comparisons test and were considered statistically significant when $P < 0.05$. In aldosterone treated and aldosterone plus spironolactone treated rats, $[Mg^{2+}]_i$ was significantly lower than in control rats. In aldosterone treated rats, $[Ca^{2+}]_i$ was not significantly different than in control rats, but that of the aldosterone plus spironolactone treated rats was lower than both the control and aldosterone treated rats. The results indicate the intracellular $[Mg^{2+}]_I$ and $[Ca^{2+}]_I$ can be measured in single cells by flow cytometry. The data are consistent with the hypothesis that in vivo aldosterone treatment disrupts the lymphocyte homeostasis of magnesium ions, but that aldosterone receptor blockade does not prevent it.

THE INVOLVEMENT OF MAC-1 IN GAMMA DELTA T CELL-MEDIATED CYTOTOXICITY. ***Janet L. Eichholz, Rupert Handgretinger, and Mario Otto,*** *Christian Brothers University, Memphis, Tennessee (JLE) and St. Jude Children's Research Hospital, Memphis, Tennessee (RH, MO).* Gamma delta T cells comprise 2–9% of peripheral T lymphocytes and exert antimicrobial and antitumor activity. Their mechanism of action comprises NK cell-like activity, and they mediate antibody-dependent cytotoxicity (ADCC). However, the mechanism of action is unknown, since most γδ T cells lack the Fc receptors CD16, CD32, and CD64. Instead, MAC-1, a β_2-integrin, is present on γδ T cells. PMN studies show that MAC-1 is involved in their adhesion to target cells in addition to the release of cytokines and superoxides. The goal of this study was to determine the involvement of MAC-1 in γδ T cell-mediated cytotoxicity. We also addressed the question whether γδ T cells produce superoxides. We performed cytotoxicity assays using γδ T cells as effectors and the human Neuroblastoma line NB1691 as targets. MAC-1 was either directly blocked with anti-MAC-1 mAb, or PI 3-kinase was blocked using wortmannin or LY294002. Humanized anti-GD2 antibody, hum14.18, was used to establish ADCC-activity by γδ T cells. To prevent FcγR involvement, mAb's directed against them were used. Oxidative burst activity of γδ T cells was determined using the stimulant phorbol myristate acetate (PMA). Superoxide production was measured by flow

cytometry using DCFH-DA. Results: γδ T cell-mediated cytotoxicity is inhibited by blocking PI3-kinase with wortmannin or LY294002. Direct cytotoxicity and ADCC were not affected by anti-MAC-1. Oxidative burst activity occurred with a correlation between superoxide production and incubation with PMA. Our results show that γδ T cells produce superoxides when stimulated with PMA. Furthermore, our findings suggest that PI 3-kinase is involved in γδ T cell-mediated cytotoxicity but the involvement of MAC-1 is inconclusive. (Supported by National Cancer Institute POE Grant 5R25 CA23944)

IDENTIFICATION OF A GENE AFFECTING CALCOFLUOR RESISTANCE, BRANCHING AND SEPTUM PLACEMENT IN *ASPERGILLUS NIDULANS*. ***Lauren M. Fay, Terry W. Hill, and Darlene Loprete,*** *Rhodes College, Memphis, Tennessee.* Cell walls define the shape of fungal cells and play important roles in mediating many interactions between cells and their environment. The assembly and modification of this complex fabric of polysaccharides and glycoproteins is incompletely understood. We have generated mutant strains of the filamentous fungus *Aspergillus nidulans* using the chemical agent NQO and have shown they contain a single gene mutation causing hypersensitivity to the chitin synthase inhibitor Calcofluor White (CFW). The phenotype of one of these strains (R205) is hyperbranched, hyperseptated and has irregular hyphal diameter. Using a plasmid genomic DNA library, we have cloned three genomic fragments that complement the mutant's phenotype. Strains harboring one of these rescuing plasmids show increased resistance to CFW and a more normal distribution of hyphal braches and septa. End-sequence analysis of each plasmid was compared to the Whitehead Institute database and the sequence of the insert was determined. Each sequence was translated and BLASTed to determine the regions that show homology to known proteins. We found two of the rescuing plasmids contain ORFs that show homology to mannose transporters. Although the nucleotide sequences are not similar in these two plasmids their translated sequences show regions of high homology to each other. Work is underway to PCR amplify the putative mannose transporters and confirm their rescuing ability.

FORCED EXPRESSION OF ACTIVATED MITOGEN-ACTIVATED PROTEIN KINASE KINASE 3 ENHANCES THE EXPRESSION OF MUSCLE-SPECIFIC PROMOTERS IN NORMAL SKELETAL MYOBLASTS BUT NOT IN RHABDOMYOSARCOMA-DERIVED CELL LINES. ***A. Frith, J. Bills, and S. Skapek,*** *Christian Brothers University, Memphis, Tennessee (AF) and Saint Jude Children's Research Hospital Memphis, Tennessee (JB, SS).* The expression of specific skeletal muscle promoters was measured in C2C12 myoblasts and a panel of rhabdomyosarcoma cell lines to determine whether ectopic expression of an activated form of mitogen-activated protein (Map) Kinase Kinase 3, known as MKK3EE, induces muscle differentiation in these cells. Each cell line was grown in culture dishes and then co-transfected with the following plasmids: 1) an expression plasmid including MKK3EE or the empty expression plasmid (pcDNA3.1), and 2) one of several reporter plasmids containing the chloramphenicol acetyltransferase (CAT) gene driven by a muscle-specific or a non-muscle-specific promoter. Transfected cells were harvested 4 or 5 days later and a CAT enzyme level was quantitated as a marker for promoter activity. MKK3EE expression induced specific muscle differentiation promoters in myoblasts but not in rhabdomyosarcoma cells. The activity was most pronounced on promoters containing DNA binding sites for MEF2, a transcription factor important for skeletal muscle differentiation. This implies that MKK3 enhances the activity of the MEF2 transcription factor. Further experiments are required to clarify why MKK3EE does not induce muscle-specific promoters in rhabdomyosarcoma cells. (Supported by POE grant 5 R25 CA23944)

HIGH COPY SUPPRESSION ANALYSIS OF MIS-LOCALIZED G1 CYCLIN *CLN3* IN THE BUDDING YEAST *SACCHAROMYCES CEREVISIAE*. ***Katherine L. Jameson and Mary E. Miller,*** *Rhodes College, Memphis, Tennessee.* Cell division involves a series of integrated, coordinated events where a single cell grows, duplicates, and segregates into two cells. *Cln3,* a G1 cyclin, integrates cellular signals initiating the cell cycle in a process that ultimately leads to division. Non-regulated division can result in cell death or uncontrollable division contributing to cancer. Little is known about the upstream regulators and downstream targets of *Cln3.* In our studies, we are utilizing a *Cln3* mutant that localizes to a non-naturally occurring cellular site. As this mutant is unable to trigger division, it is responsible for a lethal phenotype. We are investigating the molecular basis of *Cln3* activity through high copy suppression analysis wherein genes that can suppress the *Cln3*-dependent phenotype are identified. Currently, almost 40,000 transformants have been screened yielding 222 putative suppressors, which we are characterizing to identify specific genes that may be necessary for *Cln3* activity and regulated cell division.

AN OBSERVATIONAL STUDY TO REVIEW A NONINVASIVE CARDIOVASCULAR RECORDING TECHNIQUE IN PRETERM CONTRACTING PATIENTS. ***Cristina Martinez, Risa Ramsey, Lucinda Del Mar, Rosianne Mattar, and Prescilla Lindsey,*** *Christian Brothers University, Memphis, Tennessee (CM), University of Tennessee, Memphis, Tennessee (RR, LDM), and Universidade Federal de Sao Paulo, Brazil (RM, PL).* A noninvasive cardiovascular recording method that can potentially assist in monitoring changes in the cardiovascular systems of patients with symptoms of preterm labor has recently become available. The Hon cardiodynamic monitoring system (CDMS) consists of a microcomputer that connects to a pressure transducer, which is attached to the patient's finger. The microcomputer can detect and record four hemodynamic parameters including, HR, PWAT, RET, and cPP. The purpose of this study was to evaluate patients with symptoms of preterm labor using the CDMS. Previous studies conducted by Hon have shown that the CDMS has detected concomitant cardiovascular changes in patients with true labor contractions. Monitoring patients who have symptoms of preterm labor with the CDMS may provide healthcare workers with valuable insight as to which patients are in true versus false labor. The CDMS test was administered to patients upon admission to labor and delivery, and the hemodynamic parameters were assessed. Three preterm labor patients from a Latino population met the inclusion criteria and provided informed consent. The overall means and standard deviations of the CDMS hemodynamic parameters in this study were HR (83.9 ± 18.0 bpm), PWAT (137.0 ± 12.3 ms), RET (144.1 ± 13.3 ms), and cPP (14.1 ± 0.8 mmHg). The HR, PWAT, and RET were within the normal ranges previously established by Hon. The mean RET (144 ms) was higher in the enrolled contracting patients in this study than the mean RET (131 ms) previously determined by Hon on a predominantly Hispanic population of

550 patients. (Supported by NIH MIRT 1T37TW00123–03, MECF)

LOCALIZATION OF POLYAMINES DURING ATTACHMENT AND SPREADING OF RETINAL PIGMENT EPITHELIAL CELLS. ***Xuandao L. Nguyen and Dianna A. Johnson,*** *Christian Brothers University, Memphis, Tennessee and University of Tennessee, Memphis, Tennessee.* Normal cell growth and development depends upon a number of essential processes including cell attachment, spreading and migration. Disruption of these processes in retinal pigment epithelial cells (RPE) may result in blinding disorders such as gyrate atrophy and age related macular degeneration. Previous studies of the growth and development of RPE and other epithelial cell types have shown that polyamines are necessary for formation/breaking of cell attachments during migration. The purpose of this study was to determine the role of polyamines in the signaling pathways that regulate the formation of focal adhesions associated with attachment sites in RPE cells. Two different RPE cells lines (D407 and ARPE-19) were isolated and maintained in a culture medium containing Dulbecco's modified Eagles medium with bovine serum and antibiotic-antimycotic mixture. Cells were grown to confluence, fixed, and stained with antibodies against polyamines and focal adhesion kinase. Immunocytochemical staining visualized with diaminobenzidine for D407 and fluorescence for ARPE-19 cells showed the presence and localization of polyamine-immunoreactivity initially within nuclei and subsequently within vesicles during early stages of RPE cell attachment and spreading. Once cells stopped spreading and became confluent, polyamine staining was reduced. These observations suggest that polyamines are localized in nuclei of cells that are unattached or that have not begun to spread. During cell spreading polyamines are then transported from the nucleus to the plasma membranes in vesicles and subsequently released or degraded. These hypotheses may be useful in future studies to determine the mechanism involved in the cellular transport of polyamines. (Supported by The Crane Vision Research Foundation)

ANXIOLYTIC EFFECTS OF 8 OH-DPAT IN TEST-EXPERIENCED RATS ARE ABOLISHED WHEN SUBMITTED TO THE ELEVATED PLUS MAZE. ***Manish Y. Patel, Leandro J. Bertoglio, Antonio P. Carobrez, and Malinda Fitzgerald,*** *Christian Brothers University, Memphis, Tennessee (MYP, MF) and Universidade Federal de Santa Catarina, Florianopolis, SC Brazil (LJB, APC).* Previous research has shown that prior test experience concedes the anxiolytic effect of benzodiazepines (BZs) in either rats or mice, which are submitted to the Elevated Plus Maze (EPM) animal model of anxiety. This phenomenon is known as "One Trial Tolerance." Nevertheless it remains to be determined whether a similar event occurs when testing drugs that possess binding-sites on the 5-HT_{1A} receptor, such as 8 OH DPAT (a known 5-HT_{1A} agonist). We used both maze-naïve and maze experienced (free exploration of the EPM 48 h earlier for 300 sec) rats that were pretreated systemically before each trial with either 8 OH DPAT or saline and submitted to the EPM. The results confirmed the anxiolytic profile of 8 OH DPAT, represented by an increased open arm exploration and decreased risk assessment behavior in maze-naïve rats. In maze-experienced rats, however, 8 OH DPAT anxiolytic effects were not observed, suggesting that prior maze experience compromised the drug's anxiolytic activity, while increasing open arm avoidance. Thus, the "one-trial tolerance" phenomenon also might be extended to other drugs that bind to the 5-HT_{1A} receptor complex. (Supported by NIH-1T37TW00123–04)

CHYTRIDIOMYCOSIS AFFECTS LARVAL ANURAN ADAPTIVE ANTIPREDATOR BEHAVIOR. ***Elisheva Reese and Matthew J. Parris,*** *University of Memphis, Memphis, Tennessee.* Chytridiomycosis is an emerging infectious disease implicated in global amphibian declines. Pathogens may directly affect fitness by increasing mortality in their hosts, or indirectly by altering behavioral or life history traits. We exposed northern leopard frog tadpoles (*Rana pipiens*) to the pathogenic fungus *Batrachochytrium dendrobatidis* to test whether tadpole anitpredator sensory capabilities were compromised by infection. We raised tadpoles individually in laboratory containers, and exposed uninfected and infected tadpoles to visual and chemical cues from bluegill sunfish (*Lepomis macrochirus*). Containers were partitioned with a centrally-placed clear plexiglass divider. We measured tadpole activity and distance from the plexiglass in a series of treatment combinations that sequentially tested for visual and/or chemosensory abilities. Infected tadpoles had significantly lower activity levels across all treatments, and the presence of predatory cues did not alter activity levels. Infected tadpoles positioned themselves closer to the plexiglass divider than uninfected tadpoles, depending on the predatory treatment. In the presence of only visual predator cues, uninfected tadpoles oriented themselves farther from the divider than infected tadpoles. Across other treatments, uninfected and infected tadpoles showed similar orientation, suggesting that vision may be compromised by infection. Infected tadpoles may be more susceptible to predation because of attenuated visual abilities. Our results demonstrate that sublethal infection with chytridiomycosis may interact with biotic components of larval amphibian communities such as predation, potentially increasing the probability of population declines.

FUNCTIONAL MAGNETIC RESONANCE IMAGING OF ATTENTION DEFICITS IN PEDIATRIC CANCER SURVIVORS. ***John A. Sexton, Ann M. Viano, and Robert J. Ogg,*** *Rhodes College, Tennessee (JAS, AMV) and St. Jude Children's Research Hospital, Memphis, Tennessee (RJO).* Children surviving cancer or cancer therapy that affects the central nervous system are at risk for neuropsychological and cognitive impairments impacting academic performance and quality of life. Evidence from behavioral studies suggests cancer and cancer therapy induced deficits in the ability to sustain attention underlie these impairments. Functional MRI (fMRI) was used to investigate the physiological bases for these attention deficits. Subjects were school-aged (6–17) survivors (n = 24) of pediatric brain tumors or leukemia at least one year off treatment, and healthy siblings (n = 11) of the same ages. Results indicate significantly decreased volume of activation (P = 0.05) in survivors (694.29 voxels) compared to healthy siblings (1480.67 voxels). Average distances activated regions from target regions of interest were larger (P < 0.01) for survivors (10.43 voxels) than healthy siblings (5.98 voxels). These results indicate reduced volume and concentration of cortical activation in survivors compared to healthy siblings.

SALIVARY GLAND DEGENERATION IN FEMALE IXODID TICKS: NECROSIS OR PROGRAMMED CELL DEATH. ***C. K. Thompson, L. B. Coons, and Sharon Frase,*** *Christian Brothers University, Memphis, Tennessee (CKT) and University of Memphis, Memphis, Tennessee (LBC, SF).* Female ixodid ticks have

three types of acini that make up their salivary glands. After the rapid engorgement period, the salivary glands undergo degeneration. It is not yet known if this degeneration is due to necrosis or programmed cell death. Studies have shown that the degenerating cells do not fluoresce when stained with propridium iodide. This supports the belief that the degeneration is due to programmed cell death. Female ixodid ticks were fed on rabbits by methods approved by the Institutional and Animal Care Use Committee protocol. Since there is no single method in testing for programmed cell death, several methods were used. The salivary glands were dissected and prepared for examination under the Transmission Election Microscope, Scanning Electron Microscope, Light Microscope, and Confocal Scanning Laser Microscope. Salivary Glands were sectioned and stained with toludium blue, TUNEL and YO-PRO. Results show shrinkage, reduction in size of the acini, and disassembly of cytoplasmic organelles and cells. Cells stained with TUNEL are black, indicating DNA fragmentation. Cells stained with YO-PRO® fluoresce, which indicates increased membrane permeability. All characteristics of programmed cell death can be observed. Degeneration in female *Dermacentor variabilis* appears to be due to programmed cell death.

THE MITOCHONDRIAL TRANSPORTER ABCB6 PROMOTES HYPOXIC SURVIVAL AND ITS EXPRESSION IS REGULATED BY HYPOXIC SIGNALS. ***Partha C. Krishnamurthy, Guoquing Du, Tony Vu, Daxi Sun, and John D. Schuetz,*** *St. Jude Children's Research Hospital, Memphis, Tennessee (PCK, GD, DS, JDS) and Christian Brothers University, Memphis, Tennessee (TV).* Deprivation of oxygen causes metabolic adaptations within a cell to facilitate survival. One adaptation to altered oxygen levels is a change in cellular heme. We have recently demonstrated that ABCB6 is localized to the mitochondria and regulates cellular heme levels. The major transcriptional sensor of oxygen is hypoxia-inducible factor (HIF). We investigated whether HIF is involved in the transcriptional activation of ABCB6 and what functional significance this action gave to cells under hypoxic conditions. Further, the ABCB6 promoter contains hypoxia response elements (HRE) that are functional because the promoter is activated by the hypoxia mimetic, desferroxamine, as well as co-transfected HIF-1α. Moreover, in cells with a defective HIF, ABCB6 expression is not upregulated by hypoxia. Finally, ectopic overexpression of ABCB6 reveals that ABCB6 promotes cells from cytotoxicity induced by hypoxia. These studies reveal that ABCB6, a regulator of cytosolic heme levels, is regulated by hypoxia and the major hypoxia transcription factor HIF-1. This upregulation by hypoxia is compatible with ABCB6 overexpression providing a survival advantage under hypoxic conditions. (Supported by the NIGMS Grant 5 R01 GM60904-04)